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CO-INFECTION BY A SPECIES OF *PYTHIUM* AND *RHIZOCTONIA LAMELLIFERA* SMALL IN BLAST DISEASE OF OIL-PALM SEEDLINGS

By J. S. ROBERTSON

West African Institute for Oil Palm Research, near Benin City, Nigeria

(With Plate 23)

The leaf and root symptoms of blast disease are described. Isolations from roots in an advanced state of decay yielded *Rhizoctonia lamellifera* whereas isolations from primary infections yielded a *Pythium* species. Inoculation experiments are reported which demonstrate that typical blast symptoms develop only when a mixed inoculum of *R. lamellifera* and *Pythium* sp. is employed. A laboratory experiment showed that *R. lamellifera* is capable of parasitizing the *Pythium* species. Blast disease appears to result from co-infection of oil-palm seedling roots by a *Pythium* sp. and *R. lamellifera.*

INTRODUCTION

In Nigeria and other West African countries a serious root disease termed 'blast' attacks oil-palm seedlings in planted nurseries at the end of the rainy season. In infected seedlings, death results from a rapid decay of the root system. Losses of the order of 5–50 % are usual and the disease is thus a major economic problem in oil-palm cultivation.

Moureau (1952) suggested that *Thielaviopsis basicola* might be the causal organism. During investigations made prior to 1954 at the Institute's main Station near Benin, various fungi were isolated from diseased roots but none of these was known to be an active plant pathogen and all failed to produce the disease when inoculated. Studies of the nematodes associated with blast failed to show that these organisms were responsible for the disease (Bull, 1954; Robertson & Bull, 1956). Recent work has shown that root decay depends upon a co-infection of the host tissue by a *Pythium* species and *Rhizoctonia lamellifera*. The rather unusual results of these inoculation experiments are summarized in this paper.

DESCRIPTION OF THE DISEASE

The first visible symptom of blast in seedling oil palms is disappearance of the gloss characterizing the surface of a healthy leaf. The leaves become flaccid and their colour changes from dark green to olive green or khaki and then to a pale greenish yellow. Eventually purple or umber tints develop at the leaf tip and chlorosis and necrosis gradually involve the whole of the leaf surface. A rapid drying-out of the leaf ensues, followed by death.

The majority of seedlings affected by blast also show a necrosis of the

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youngest unexpanded leaf. This symptom may appear at the tip of the leaf or it may affect only the basal tissue. A species of *Fusarium* has been isolated from this necrosed tissue, but its significance in the aetiology of the spear rot is obscure.

Root infection occurs at the tips of the fleshy primary roots. The parenchymatous tissue within the hypodermis is rapidly destroyed, and as the disease proceeds the rot progresses along the root until it reaches the definitive stem tissue or 'bulb' where it is arrested. There is no evidence of a physical barrier to infection of the bulb and resistance of this tissue to infection may be chemical in nature.

Blast is fatal to about 95 % of all seedlings attacked. Those which recover are invariably reduced in size and vigour and are unsuitable for planting out.

ISOLATIONS FROM DISEASED ROOTS

Rhizoctonia lamellifera Small. This fungus has been directly isolated from cortical and vascular tissues in an advanced state of decay. Sclerotia of the fungus are present in the degenerating cortical tissue behind the transition zone; at a later stage, when the root cortex has been completely destroyed, small black sclerotia can be observed on the naked stele. *R. lamellifera* grows rapidly on artificial media, producing a white hyaline appressed mycelium which becomes olive green and finally black, as abundant sclerotia (0·75–1·00 mm.) are produced.

Pythium species. This fungus can be directly isolated only from primary infections of the fleshy root tips. At a more advanced state of root decay it is possible to demonstrate the presence of this fungus by means of a washing technique (Hopkins, 1956). Small pieces of infected root tissue are placed in a muslin bag and suspended in a stream of running tap water for 24 hr. The pieces of washed root are then cut into smaller pieces and placed in sterile water. After a further period of 24 hr., *Pythium* mycelium begins to grow out of the tissue and numerous sporangia are produced. Pieces of infected tissue placed in sterile water without previous washing produce a mass of secondary, saprophytic fungi, together with *R. lamellifera*. The reason for the difference in behaviour after washing is obscure, but the technique has proved valuable in isolating the *Pythium* sp. from mixed infections.

The *Pythium* sp. isolated from blast grows well on potato-dextrose agar, producing a white appressed mycelium and abundant sporangia, measuring 20–25 μ. The latter show a behaviour different from that in most species of *Pythium*, and germinate by means of a germ tube. The failure to observe sex organs and the production of oospores has made final identification of this species impossible.

EARLY INOCULATION EXPERIMENTS

With Rhizoctonia lamellifera. Seedlings were inoculated by dipping roots in an inoculum prepared by macerating vigorously growing cultures of *R. lamillifera* in sterile water in a MSE homogenizer. It was found impossible to infect undamaged roots, but roots artificially damaged prior to inoculation, by scraping with a scalpel until the cortex was exposed, developed a cortical rot from which the fungus could readily be re-isolated. Typical blast leaf symptoms did not develop, the most prevalent leaf symptom being a black 'wither tip' condition unlike blast symptoms as seen in the field.

With the Pythium *species.* All seedlings inoculated with a *Pythium* sp. macerate prepared as above developed a typical cortical root rot. In many seedlings, infection was confined to small areas of the root tips. The leaf symptoms produced were variable but in general affected seedlings showed incipient wilt of the leaves, which became dull and flaccid. Necrosis of the leaves was occasionally observed but most of the inoculated seedlings ultimately recovered.

PARASITISM OF *PYTHIUM* SP. BY *RHIZOCTONIA LAMELLIFERA*

Although the above inoculation experiments showed that leaf symptoms could result from invasion of the primary roots by either *Pythium* sp. or *R. lamellifera* when artificially inoculated, the symptoms produced were atypical and the chlorosis and umber tints, characteristic of leaves of diseased plants in the field, were not observed.

At this point in the investigation, the literature was searched for records of an association between species of *Rhizoctonia* and *Pythium*. A paper by Butler (1957) described experiments which showed that *R. solani* could actively parasitize *P. splendens* when the two fungi were grown in mixed culture. Butler's experiments were therefore repeated, using *R. lamellifera* and the *Pythium* sp. isolated from blast.

Plates of potato-dextrose agar were inoculated on opposite sides with vigorously growing cultures of the two fungi and incubated at 29° C. The first examination was made some 36 hr. after the two colonies had become intermingled, and subsequent examinations were made at intervals of 24 hr. At the junction of the two colonies, *R. lamellifera* was seen to produce a dense aerial growth and parasitism of the *Pythium* sp. was first observed in this region. In the earliest stage of parasitism, there was an active coiling of septate *Rhizoctonia* hyphae around the much broader coenocytic *Pythium* hyphae (Pl. 23, fig. 1). No penetration of *Pythium* hyphae was observed at this stage. In one instance, the *Rhizoctonia* had produced small structures resembling infection pegs which were appressed to the *Pythium* mycelium, but it was not possible to ascertain whether or not these had actually penetrated the host hyphae.

After approximately 72 hr., direct penetration of the *Pythium* hyphae occurred, but this stage of attack was not associated with the coiling of *Rhizoctonia* mycelium previously described (Pl. 23, fig. 2). After entry into a *Pythium* hypha, the invading *Rhizoctonia* hyphae developed rapidly, causing an immediate lysis of the cell contents, which rapidly disappeared. In several instances, small peg-like haustoria were produced within host hyphae after the *Rhizoctonia* had established penetration.

Penetration and parasitism of *Pythium* sporangia occurred as soon as the latter were formed. There were two methods of penetration:

(*a*) Direct penetration (Pl. 23, fig. 3), similar to that found when hyphae were invaded.

(*b*) Multiple penetration, where the *Rhizoctonia* hyphae became grouped to form a cluster of appressoria on or around a sporangium prior to infection (Pl. 23, fig. 4).

In both types of invasion, the *Rhizoctonia* ramified freely within the sporangium, once penetration had occurred. The cytoplasm within invaded sporangia disintegrated and disappeared, and ultimately the sporangia collapsed completely.

In none of the experiments did *R. lamellifera* succeed in parasitizing and killing the whole of the *Pythium* culture. Isolations from mixed cultures always produced both *R. lamellifera* and *Pythium* sp. The *Pythium* sp. appeared to have no resistance, either of a chemical or a physical nature, to infection by *R. lamellifera*. Physical barriers to invasion, such as newly formed cross-walls, did not appear in parasitized *Pythium* hyphae, nor did the invading *Rhizoctonia* hyphae become necrosed or in any way changed.

FINAL INOCULATION EXPERIMENTS

Using a mixed inoculum of Pythium *and* Rhizoctonia. Seedlings were inoculated by dipping the roots in a mixed inoculum prepared by macerating ten cultures of the *Pythium* sp. and ten cultures of *R. lamellifera*. All inoculated seedlings developed typical root and leaf symptoms of blast within 7 days of planting. Isolations from roots in an advanced state of decay yielded *R. lamellifera*, but it was also possible to demonstrate the presence of the *Pythium* sp., using the washing technique already described.

DISCUSSION

There appears to be some controversy as to whether *R. lamellifera* is an active pathogen or not. Some workers, including Small (1927, 1928*a*, *b*), considered that *R. lamellifera* is an active parasite, capable of penetrating healthy roots and causing a rapid degeneration of both cortical and vascular tissue. Others, in particular Briton-Jones (1928), asserted that the organism is a facultative root parasite, and that penetration into the host is dependent upon certain conditions predisposing the host to infection. Yet others were of the opinion that the fungus is a secondary parasite, or even a saprophyte.

Inoculation experiments have shown that this fungus is not able to penetrate healthy, undamaged roots. It is possible, however, for the fungus to gain access to the internal root tissue through lesions caused by mechanical damage, but one would not expect this to be widespread under normal conditions in oil-palm nurseries. If mechanical damage is artificially produced on the roots of seedlings planted in inoculation trials, then infection does take place and degeneration occurs, but typical leaf symptoms of blast are not produced.

In blast disease, *R. lamellifera* obviously plays an important role in the destruction of cortical tissue, and it is now thought that this fungus

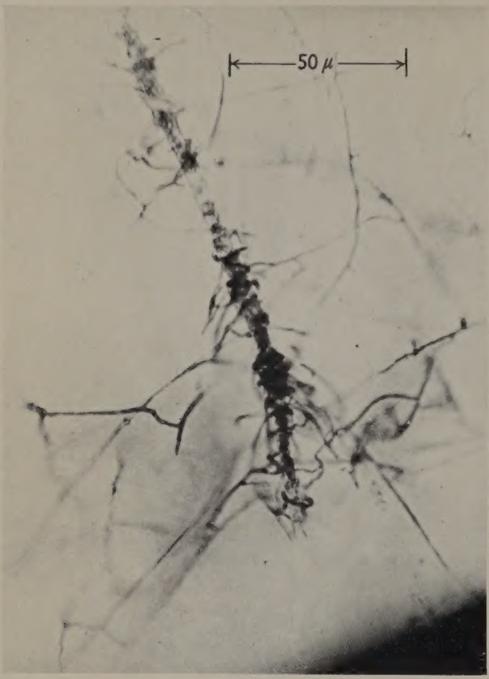

Fig. 1

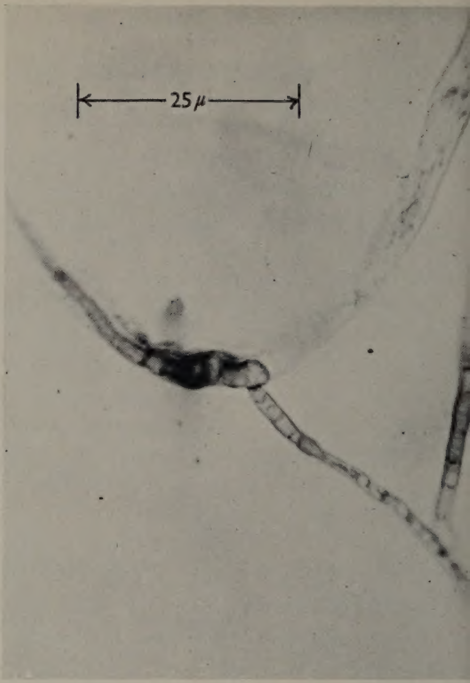

Fig. 2

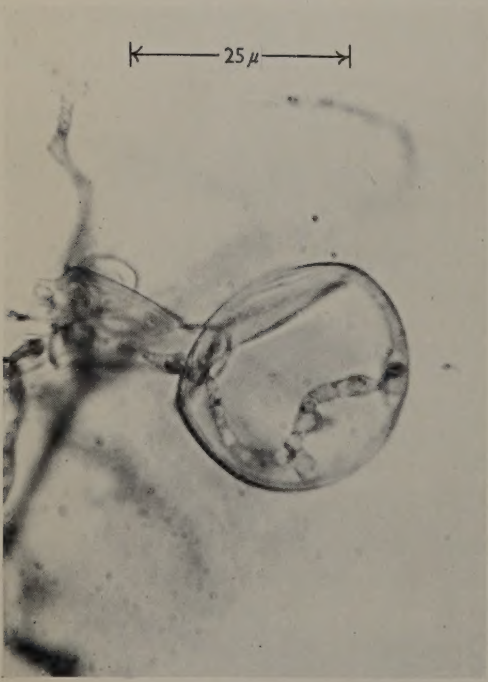

Fig. 3

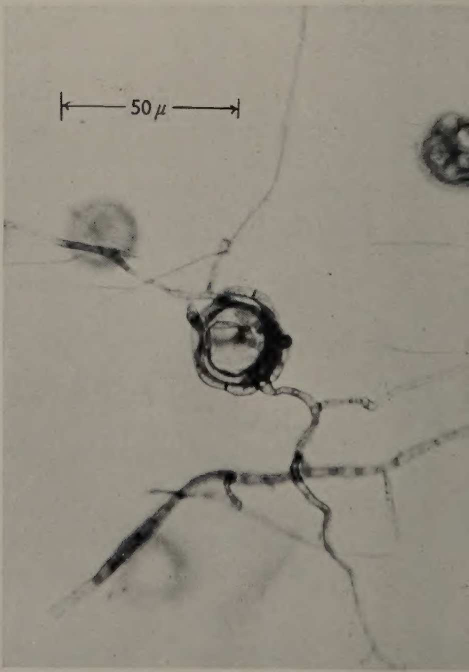

Fig. 4

(*Facing p.* 405)

establishes itself by parasitizing the *Pythium* species, the latter fungus being the primary pathogen. This hypothesis is supported by the evidence of laboratory studies on the parasitism of the *Pythium* by *R. lamellifera*. Results of the inoculation trials show that the *Pythium* sp. associated with blast disease is the primary pathogen. The *Pythium* sp. is capable of penetrating healthy undamaged roots; once it has gained access, it produces total necrosis of the cortical and vascular tissues. The point of penetration usually occurs at the growing points of the root, but rate of growth of the fungus within the host tissue is not as high as that of *R. lamellifera*. This may be because the *Pythium* sp. grows intracellularly, with much of the mycelial development occurring within the cytoplasm of cortical cells.

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EXPLANATION OF PLATE 23

Fig. 1. First stage in the parasitism of *Pythium* sp. by *Rhizoctonia lamellifera*, showing *Rhizoctonia* hyphae coiling round the *Pythium* hypha.

Fig. 2. Penetration of *Pythium* hypha by hypha of *R. lamellifera*.

Fig. 3. Direct penetration of *Rhizoctonia* hypha into a *Pythium* sporangium.

Fig. 4. Multiple penetration. The *Rhizoctonia* hyphae have become grouped to form a cluster of appressoria around the *Pythium* sporangium, prior to penetration.

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PHYMATOTRICHUM FIMICOLA SP.NOV., A COPROPHILOUS HYPHOMYCETE

By VIVIENNE J. DRING

(With 1 Text-figure)

Phymatotrichum fimicola sp.nov. (Fig. 1)

Mycelium ex hyphis ramosis, septatis, hyalinis compositum. Conidiophora primo hyalina dein brunnea, septata, 0·5–0·75 cm. longa, 20–30 μ crassa, basim versus crasse tunicata, apice ramosa. Rami 4–5 dichotomi, divergentes, apicibus sterilibus acutiusculis. Conidia (radulospora) ex lateribus ramorum in sterigmatibus spiraliter gerentes, spherica, primo hyalina, levia, dein brunnea, sulcata, 11–15 μ diam.

Hab. in stercore muris, Aburi, Ghana, xi, 1957. Herb. I.M.I. 73530 typus.

In October 1957 an interesting fungus appeared on mouse dung which had been collected about 10 days previously at Aburi, Ghana. It was isolated in pure culture and grown on horse-dung agar, oatmeal agar and cassava dextrose agar.

The original collection consisted of conidiophores up to 0·75 cm. long, in divergent groups (Fig. 1*a*); they were unbranched in the lower region but dichotomized repeatedly at the apex to form a ball (Fig. 1*b*). The base of the conidiophore was thick walled, ending abruptly just below the surface of the dung, and had a weft of very fine hyphae arising from it (Fig. 1*c*).

The branches at the top of the conidiophore were in a spiral, and each dichotomized four or five times, the two most distal branches bearing spores (Fig. 1*d–e*). The spores arose spirally on the branches of the conidiophore on short sterigmata (Fig. 1*f–g*). They were hyaline and smooth when first formed, but later became lightly ridged and brown. Dispersal occurred mainly through the detachment of the ultimate branchlets and not by the detachment of individual spores. The conidiophore showed marked hygroscopic movement, and when examined in air the head revolved as the conidiophore dried.

The conidiophores were pure white when first formed, but at maturity they quickly changed to buff and finally, with age, to brown.

On cassava dextrose agar *P. fimicola* reaches a much darker colour than when grown on oatmeal or dung agars. In tubes sporulation tends to occur on the glass, and in Petri dishes mostly round the edge of the plate, the conidiophores usually arising singly and not in the characteristic groups found in nature. In culture, at tropical laboratory temperature, sporulation occurs 5–10 days after inoculation.

This species differs in several major respects from the original description of the genus *Phymatotrichum* Bonorden (*Handbuch der allgemeinen Mykologie*, p. 116, 1851) and from all hitherto described species of *Phymatotrichum*.

The terminal branches of the conidiophore are not swollen as in other species, but have an extensive, sterile, pointed tip (Fig. 1 *g*), and the overall form of the conidiophore is not tree-like or bushy, but more like a round-headed pin (Fig. 1 *a*). However, the fungus is very near *Phymatotrichum* and at present there do not appear to be sufficient grounds for erecting a new genus, though the discovery of other very similar species might alter this view.

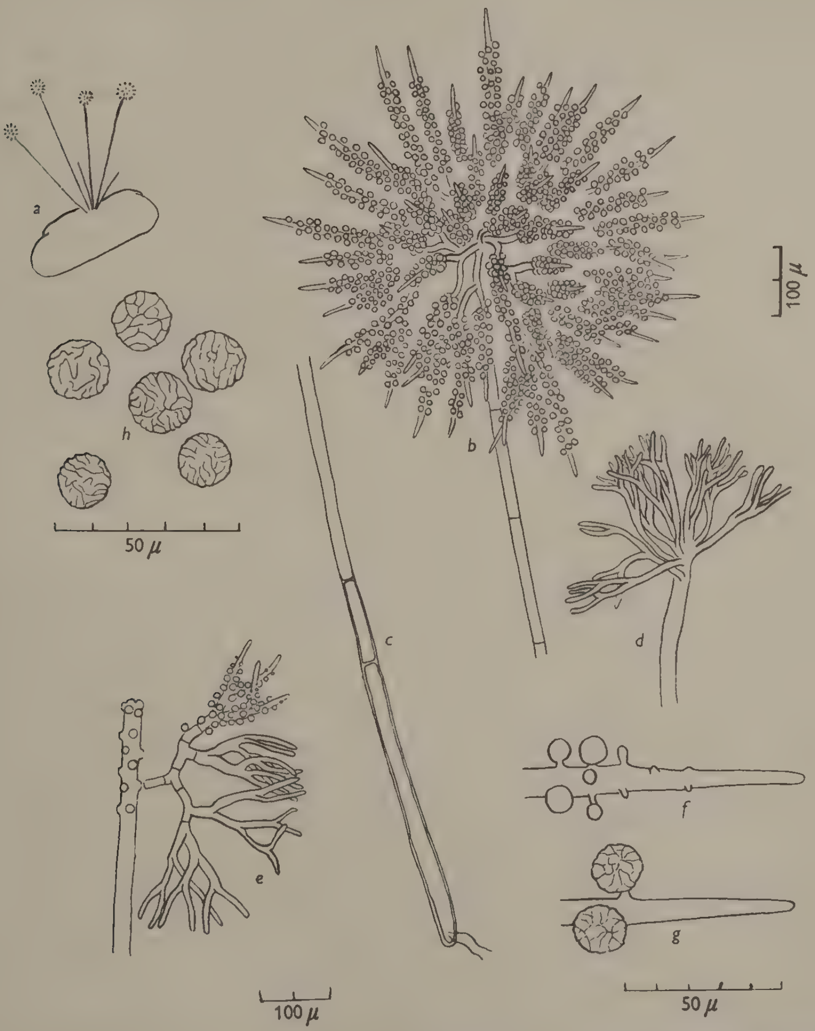

Fig. 1. *Phymatotrichum fimicola. a*, conidiophores growing on dung; *b*, head of conidiophore; *c*, base of conidiophore; *d*, immature conidiophore; *e*, part of conidiophore to show dichotomy; *f*, immature spores on terminal branch and sterigmata; *g*, mature spores on terminal branch; *h*, mature spores.

The author wishes to express her thanks to Prof. C. T. Ingold and Dr M. B. Ellis for their kind help, and to Sr. Marie Immaculata for help with the Latin diagnosis.

Cultures and slides have been deposited at the Commonwealth Mycological Institute, Kew.

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THE POSSIBLE ROLE OF PATHOGENIC FUNGI IN DIE-BACK OF *SPARTINA TOWNSENDII* AGG.

By P. J. GOODMAN*

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A recession of *Spartina townsendii*, known as die-back, in mud flats in the Hampshire basin, showed symptoms inconsistent with attack by a primary pathogen. Soft-rotted rhizomes of die-back plants contained no bacteria or fungi until rotting was well advanced, and an examination of moribund material revealed no obviously pathogenic fungi.

Seedlings planted in sand culture alongside heavily fungus-infected plants grew strongly, and unhealthy plants set beside healthy plants in the field did not pass on the infection to the healthy ones, but themselves recovered. On the other hand, healthy plants transplanted to die-back sites died. It was concluded that attack by a strong primary pathogen was not involved, and that the cause of the disease was essentially non-parasitic. The association of the die-back with certain definite types of site emphasized the need for an investigation of the ecological conditions governing occurrence of the disease.

INTRODUCTION

Recession of the marine grass *Spartina townsendii* from the mud flats along the channel sides of estuaries in the Hampshire Basin was first reported around 1930. This recession, characterized by local unhealthy appearance and final death of plants, became known in the area as 'die-back', and was believed indirectly to cause silting-up of the river channels. At the request of British Railways, owners of the ferry which runs from Lymington to Yarmouth, Isle of Wight, a brief survey of die-back was made by Austwick (1950). This survey revealed not only die-back along the channel edges, but also in the sward in the 'pans' which hold water between the tides.

Spartina plants collected by Austwick (1950) from the edges of die-back sites bore no fungus known to be, or on general grounds likely to be, a serious parasite, and showed no specific symptom of attack. Nevertheless, in the course of a full investigation of die-back (Goodman, 1957), a more extensive study was made of the possibility of pathogen attack on *Spartina*, since Austwick's survey had been inconclusive in this respect. Although it had been suggested that pathogens other than fungi were unlikely to be involved, further search for possible fungal pathogens was considered necessary.

On *S. townsendii* in healthy sward in Britain, only two fungi, *Didymosphaeria spartinae* and *Hendersonia culmicola* (Grove, 1933, 1935–7) were recorded before Lloyd (1952) collected eight species in the Dovey estuary. None was found on living plants, but dead and decaying stems and leaf sheaths often bore mycelium and fruit-bodies. Pycnidia and perithecia of several pyrenomycetes and Fungi Imperfecti occupied the air chambers of the stems. Stems on the drift line bore apothecia of a discomycete.

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EXAMINATION OF FIELD MATERIAL

Collections of *Spartina* material were made every 2 months at Lymington and elsewhere between August 1953 and August 1954. The morphology and anatomy of healthy and die-back plants were compared, and the collections were examined for the presence of possible pathogens.

SYMPTOMS OF DIE-BACK

On die-back edges there were many dead plants and such living plants as were present were in a moribund state. They were yellowish, with rather few, weak tillers and tiller buds, and failure of these brought about a continual increase in the amount of dead material at the expense of the live material of each plant.

By far the most characteristic symptom of die-back was rhizome failure. While healthy rhizomes were strong and turgid, containing abundant starch, die-back rhizomes were weak and flaccid, with enlarged air chambers and little starch in the watery tissues. There was a very weak point just behind the vegetative apex, where the air chambers began to form.

Material which was actually alive and growing at the time of collection was rarely infected by other than ?*Ligniera* sp. This species was found in healthy plants but not in those with die-back symptoms. No fungi or bacteria were associated with weak though still living die-back material, but as the course of die-back progressed, rhizomes and roots became soft-rotted, all but the tougher tissues being destroyed. Bacterial infection occurred in the advanced soft-rotting stage. Eventual rotting, after secondary infection, left only the lignified elements of the vascular and cortical tissues of the rhizomes. Stages comparable to those found late in the soft-rotting process were noted by Burkholder & Bornside (1957) in decomposition of cut *Spartina alterniflora* stems by marine saprophytic bacteria. Apparently the late stages of rotting seen in *S. townsendii* were normal for the habitat, though in one exceptional case a barrier layer was seen, bacterial infection having occurred in a live rhizome.

There was no sign of general primary pathogen attack, either by fungi or by other organisms, though fungal mycelium was occasionally found, near the point of damage on leaves torn from the sheaths, in living plants near the estuary mouth. These plants with torn leaves showed no die-back symptoms.

FUNGI ISOLATED

Dead material examined consisted of (*a*) the lowest stem nodes, the air chambers of which contained perithecia and pycnidia, (*b*) soft-rotted roots and rhizomes. Wherever possible, fungal fruit-bodies were identified.

The *Spartina* material was then surface-sterilized and cultured. For surface sterilization, three similar samples were (*a*) flamed, (*b*) treated with 0·1 % mercuric chloride solution, (*c*) treated with 2 % sodium hypochlorite solution, respectively. Pyrenomycetes and Fungi Imperfecti were isolated and cultured from single asci or from spores, from the surface-sterilized fruit-bodies. A range of culture media was used, including Czapek-Dox agar, potato agar, potato-dextrose agar, and oatmeal agar, made up both with distilled water and with sea water, respectively. The following fungi were isolated and identified:

PLASMODIOPHORALES
?*Ligniera junci* (Schw.) Maire & Tison—in living roots.

PHYCOMYCETES
Rhizopus nigricans Ehrenb.—on old stems.

ASCOMYCETES
Didymosphaeria spartinae Grove—on old stems.
Amphisphaeria culmicola Sacc.—on old stems, lower river, abundant February.
Leptosphaeria typharum (Desm.) Karst.—on old stems.
Pleospora salsolae Fuckel—on old glumes and raches, abundant November.
Lulworthia medusa (Ellis & Everh.) Cribb & Cribb—on oldest parts, upper river only.

FUNGI IMPERFECTI
Cladosporium herbarum Link ex Fr.—on old stems.
Botrytis cinerea Pers. ex Fr.—on old stems.
Alternaria maritima Sutherland—on stems of various ages.
Penicillium spp.
Aspergillus spp. } On old parts, fairly frequently.
Phoma sp.—surface mycelium commonly present all year round.
Cephalosporium sp.—on one culture of old leaves.
Paecilomyces sp.—on one culture from pan die-back.
Acrothecium sp.—on one culture of roots.
Coniothyrium sp.—on one culture from pan die-back.

All except ?*Ligniera* were isolated and grown in culture. A number of cultures remained sterile and were therefore unidentifiable. Specimens of the less common species have been deposited in the Commonwealth Mycological Institute, Kew, as were those found by Austwick (1950).

The fungi listed above cover all those collected by Austwick, with the following exceptions:

Phytophthora sp.
Pythiogeton sp. }—on excised leaves in culture.
Chaetomium cochloides Palliser—in one moist culture of dead leaves.
Epicoccum purpurascens Ehrenb.—in one moist culture of dead leaves.
Volutella ciliata (Alb. & Schw.) Fr.—in a moist culture of rhizomes.

Leaves were commonly covered with an algal felt which possibly supported the Phycomycete species which Austwick isolated.

No obvious parasites were isolated from the surface-sterilized material. ?*Ligniera*, which alone inhabited living plants, occurred in both healthy and die-back collections, and apparently caused no hypertrophy. Absence of hypertrophy is characteristic of the genus (Cook, 1926). The spore size of the collected material differed slightly from that of *L. junci*: without full knowledge of the life history this organism cannot be identified with certainty. *Amphisphaeria* and *Lulworthia* might, from their systematic positions, well be pathogenic, but the fact that neither occurred over the full range of die-back sites in both upper and lower reaches of the estuary excludes them from serious consideration. Moreover, *Lulworthia* has been reported on plants lacking die-back symptoms, in areas completely free from die-back (Lloyd, 1952).

Since the primary object of the investigation was to discover pathogens, no attempt was made to identify critically species considered to be certain saprophytes. The roles of unidentified fungi which might be parasitic, and of *Stagonospora* sp. and *Phoma* sp., belonging to genera including both saprophytes and parasites, could only be determined by inoculation experiments.

INOCULATION, RECOVERY AND TRANSPLANT EXPERIMENTS

Inoculation experiments

To determine whether fungal disease could be transferred from dying to healthy plants, simple small-scale inoculation experiments were carried out. In the first of these, three heavily fungus-infected, partly living plants were set beside healthy plants in pots of a sand–soil mixture. The infection was not passed on, and the infected plants recovered to some extent.

In a second experiment, chopped, fungus-containing *Spartina* material was introduced into sand in two pots in each of which twenty-four *Spartina* seedlings were growing. The seedlings failed to become infected.

In a third experiment, two heavily fungus-infected plants were set in pots of sand in which *Spartina* seedlings were growing. The seedlings not only failed to become infected, but a most striking change occurred in the moribund introduced plants. These plants grew strongly, tillering and flowering vigorously in the sand cultures.

Recovery experiments

The recovery effect, on bringing heavily fungus-infected plants from channel die-back sites and from pan die-back sites into sand culture, was thoroughly established by experiment. The results of the first experiment, recording the growth of plants from channel die-back sites before and after a year's culture in sand supplied with culture solution, are given in Table 1. A second similar experiment, using plants from pan die-back areas, gave comparable results.

These results indicate that the recovery of fungus-infected plants brought in from the edges of die-back sites is a general phenomenon.

Table 1. *Recovery of* Spartina townsendii *on transplanting from die-back sites at Lymington to sand cultures*

	Beginning of experiment (September 1954).	End of experiment (September 1955)		
	No. shoots	No. shoots	No. inflorescences	No. rhizomes
King's Post	11	26	2	12
Pier	13	28	1	7
No. 11 Post	5	7	0	4
Tar Barrel	7	24	6	4
No. 5 Post	13	32	4	8

Transplant experiments

When it became reasonably certain that no general infection would occur as a result of introducing fungus-bearing plants into healthy sward areas in the field, transplant experiments were begun (Table 2). In the first series (Table 2 *a*), moribund plants were transferred from channel and pan die-back edges to healthy sward. Of six depauperate plants transferred from channel die-back in the Lymington Pier area to a healthy site near Harper's Post, three recovered completely, becoming indistinguishable

Table 2. *Transplant experiments*

		Live plant numbers		
Source of plants	Site on which planted	Beginning of experiment	End of experiment (one year later)	Die-back site condition
	(*a*) Die-back plants in healthy sites			
Lymington Pier	Lymington, Harper's Post	6	3	Severe channel die-back
Lymington, Cocked Hat pan	Lymington, Cocked Hat sward	24	21	Severe pan die-back
	(*b*) Healthy plants in die-back sites			
Beaulieu	Beaulieu pan	6	0	Severe pan die-back
Havant	Havant Pook	6	0	Severe channel die-back
Poole (Sandbanks)	Lymington:			
	Walhampton	125	0	Severe channel die-back
	King's Post	250	0	Severe channel die-back
	Pier	250	2	Severe channel die-back
	No. 11 Post	250	0	Severe channel die-back
	Tar Barrel	250	8	Erosion, some die-back
	No. 5 Post	125	3	Severe erosion, little die-back
Lymington, Cocked Hat sward	Cocked Hat pan	12	0	Severe pan die-back

from the nearby sward; a fourth partially recovered, while the remaining two were apparently too badly affected to recover. Of twenty-four plants transferred from pan die-back near Lymington Cocked Hat to a nearby healthy area, twenty-one plants recovered completely.

In the second series of transplant experiments (Table 2*b*), the reciprocal operation of introducing healthy plants into die-back areas was carried out. Cuttings were taken from healthy sward by the method of Carey & Oliver (1918). A test transplant of twelve plants into a healthy sward area completely denuded in a freak storm showed that, in suitable areas, the cuttings were 100% viable, growing strongly and flowering in one year. Yet, of 1250 similar cuttings set in channel die-back sites at Lymington, 100% died in up-river sites, with no symptoms other than complete failure in rhizome and tiller production. In the lower river, 3% survived, producing dwarfed plants with small, often torn, leaves similar to those of the living plants nearby, but differing from those upstream. Failure in the lower river was apparently due in some measure to wave action, and other evidence supported this (Goodman, in preparation).

CONCLUSIONS

From the evidence presented, it will be seen that the symptoms of die-back are inconsistent with general primary pathogen attack. The characteristically soft-rotted rhizomes of die-back plants contained no bacteria or fungi until a late stage in the course of die-back was reached. No obviously pathogenic fungi were isolated from moribund material.

In the pot inoculation experiments, seedlings in sand culture alongside heavily fungus-infected plants grew strongly, tillering and flowering. In the recovery experiments, unhealthy plants set beside healthy ones in the field did not pass on infection, but themselves recovered in the 'healthy' conditions. These results would not be expected if attack by a strong primary pathogen were involved: they indicate the importance of the environment of the plant in relation to the development of die-back.

The transplant experiments showed conclusively that die-back was associated with definite sites, in which introduced healthy plants died. This very marked 'site effect', taken together with the other results, emphasized the need for an investigation of the general ecological conditions governing *Spartina* die-back, and this investigation will be described elsewhere (Goodman, Braybrooks & Lambert, 1959).

Dr J. G. Manners gave substantial advice throughout the investigation. Dr I. Wilson assisted with identifications. My thanks are due to them, and also to Sir Edward Salisbury and Mr P. K. C. Austwick for permission to quote from the latter's original report. The work was made possible by an Assistantship awarded by the Agricultural Research Council, which is here gratefully acknowledged.

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TRICELLULA AQUATICA SP.NOV., AN AQUATIC HYPHOMYCETE

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(With 2 Text-figures)

Spores of a new species of *Tricellula* have been seen from decaying submerged leaves. The fungus has been grown in culture, and its morphology is compared with *T. inaequalis* and *T. curvatis*, two previously described species known only from cultures derived from air-borne spores.

Leaves of alder (*Alnus glutinosa*) and chestnut (*Castanea sativa*) collected from the bed of the River Porter, Sheffield, on 4–6 Oct. 1958, were kept in Petri dishes of water in the laboratory. Two days later spores of a species of *Tricellula* were discovered in the water, but spores were not seen actually attached to the leaves. Cultures were prepared by taking up spores in a fine capillary pipette and plating on 2 % malt-extract agar. The spores germinated overnight and the cultures grew slowly (5 mm. diam. in 14 days at room temperature). The description below is based on the spores from the submerged leaves, and from a 14-day-old culture on malt-extract agar. For descriptions of the fungus on other media see Table 1.

SPORES FROM LEAVES

Three-celled, consisting of a basal cell, which bears at its tip two further cells each separated by a constriction from the basal cell. One of the two upper cells continues the axis of the basal cell, but the second is produced laterally, and is slightly shorter than the first. The basal cell measures 5–6 × 2–3 μ, and the upper cells 6–8 × 2–3 μ. The total span of the spore is 11–15 × 6–10 μ (Fig. 1A).

CULTURES

Colonies discrete, slimy, white or yellowish, heaped up in the centre. Mycelium hyaline, septate, composed of inflated cells, up to 4 μ wide, constricted at the septa. The conidia arise at the tips of inflated segments of branches which may be single or in clusters. The conidium arises by budding and first forms a single ellipsoidal cell. Later a bud arises at the tip of this cell and continues growth in the same direction as the parent cell from which it is separated by a narrow constriction. A second bud arises laterally to the first and this continues to extend upwards, but curves outwards. The shape of the upper cells varies: they are constricted at the base, wider near the middle and then taper to a rounded tip, and may be slightly curved away from the axis of the basal cell. In

Table. 1. *Comparison of growth and spore measurements of* Tricellula *spp.*

	Growth after 4 weeks at room temperature on				Dimensions of spores (μ) on oat agar			
	Malt	Maize	Potato dextrose	Oat	Overall	Cell 1	Cell 2	Cell 3
T. inaequalis	2 cm., pink above and below, with radial furrows, aerial mycelium, fluffy, slightly aggregated sporulation sparse	1 mm., white to pale pink, yeast-like, spores not found	2·8 cm., pink to orange, corrugated, sparse aerial mycelium, sparse sporulation	3 cm., pale pink with aerial mycelium aggregated into prominent strands, good sporulation	8–12 × 5–6	4·5–5·5 × 2–2·2	3·5–6·5 × 2–2·2	3·5–6·5 × 2–2·5
T. curvatis	2 cm., pale pink above, buff below, with radial furrows, aerial mycelium forming prominent strands, sporulation sparse	1 cm., restricted white, flat; spores mostly 2-celled	2 cm., flat, radially furrowed, orange above and below, spores not found	3 cm., orange to pink, with aerial mycelium aggregated into prominent strands; sporulation abundant	5·5–9 × 5·5–6	3·5–4 × 1·8–2	2·7–5 × 1·8–2	2·7–4 × 1·8–2
T. aquatica	2 cm., orange to white, with radial folds and a much convoluted upper surface, aerial, mycelium absent, sporulation abundant	3 cm., flat, pale yellow to orange, aerial mycelium absent, sporulation abundant	2·5 cm., orange with radial furrows and a convoluted upper surface, aerial mycelium absent, sporulation abundant	2·5 cm., orange, showing concentric zonation, flat, aerial mycelium absent, sporulation abundant	9–12 × 5–6	4·5–5 × 2·2–2·5	4·5–5 × 2–2·2	4–5 × 2–2·2

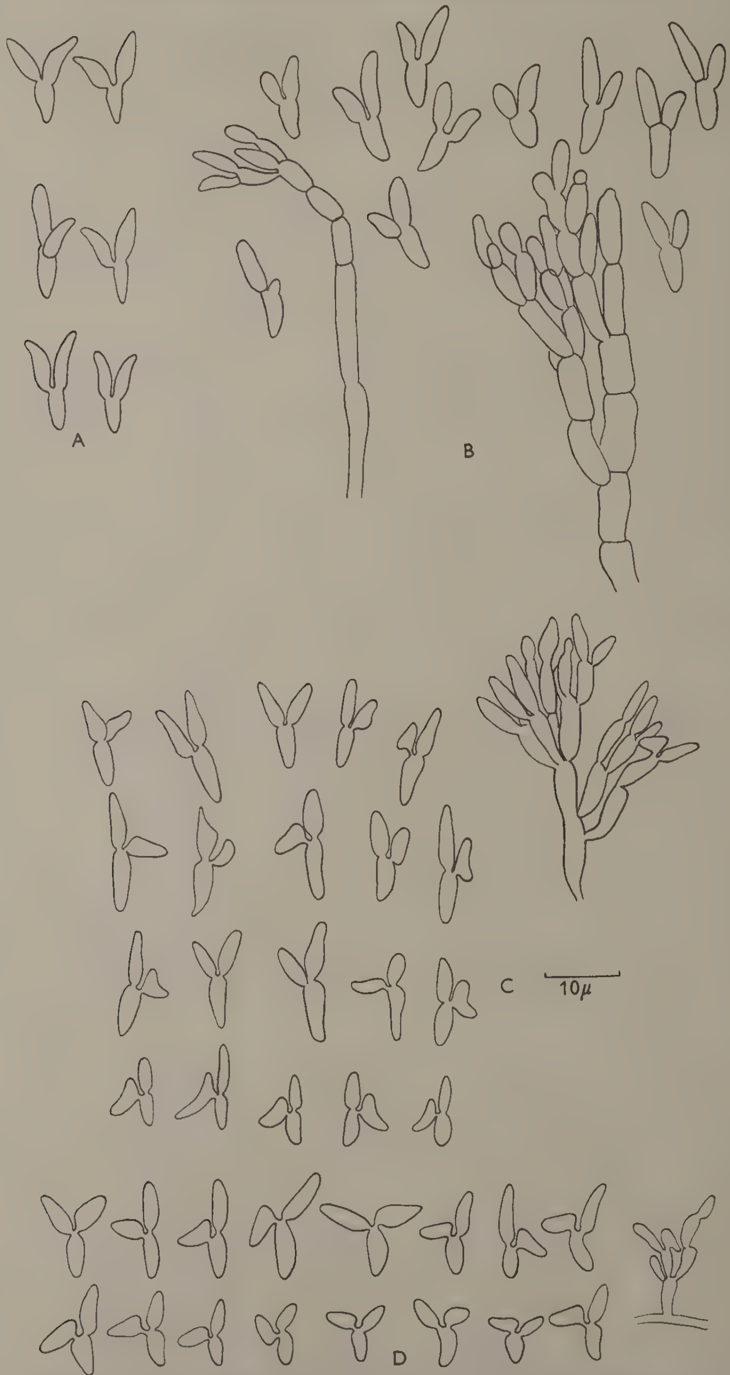

Fig. 1. A, *Tricellula aquatica*: spores from a submerged leaf of *Alnus glutinosa*; B, *T. aquatica*, spores and conidiophores from a 14-day culture on 2% malt-extract agar; C, *T. inaequalis*, spores and conidiophores from culture; D, *T. curvatis*, spores and conidiophores from culture.

some spores one or both of the upper cells may be separated from the basal cell by a septum, but this is not always present. Spores from the culture measured 9–12 × 5–6 μ.

The genus *Tricellula* is based on *T. inaequalis* van Beverwijk (1954), first isolated on an agar plate exposed to the air at Bergen in Norway. Gordon (1956) claimed to have isolated the fungus from air near Atlanta, Georgia, U.S.A., but Haskins (1958) has shown that Gordon's isolate differs from *T. inaequalis* and he has named it *T. curvatis*. The isolate from submerged leaves has been compared with both these species on a range of culture media and it is distinct. The differences between the three cultures are tabulated above. The shape of the spore is probably the most useful diagnostic feature. In *T. inaequalis* a characteristic feature is the curved *backwardly directed* growth of the lateral cell. In *T. curvatis* the three cells, due to curvature, are arranged more or less symmetrically with the three arms separated by an angle of 120°. In *T. aquatica* the spore is roughly Y-shaped. There are also differences in dimensions and these are indicated in the table. The differences are shown in Fig. 2, a tracing from enlarged photomicrographs at a magnification of 5000.

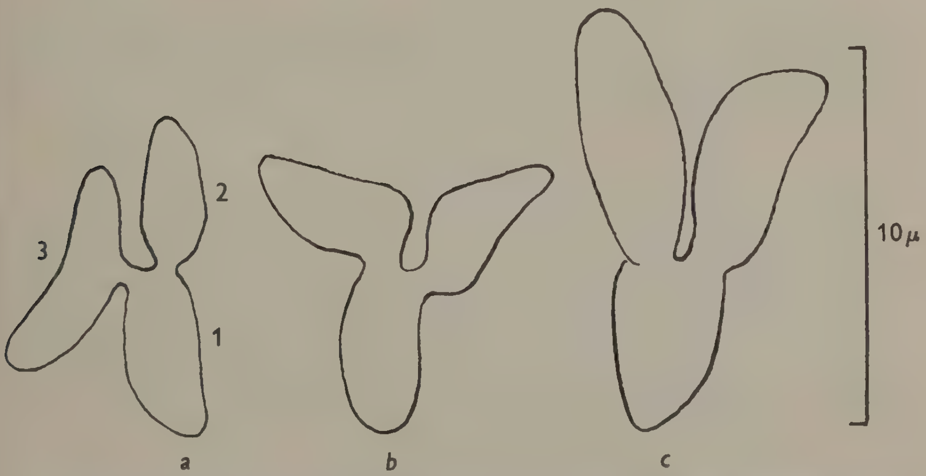

Fig. 2. Spores × 5000 of (*a*) *Tricellula inaequalis*, (*b*) *T. curvatis*, (*c*) *T. aquatica*, all from 1-month-old cultures on oat agar. The numbers of the cells on the spore of *T. inaequalis* show the convention followed in Table 1 for giving dimensions of the cells of the spore.

Van Beverwijk and Haskins state that the spores arise from phialides, but I have been unable to satisfy myself that this is true in any of the three species studied.

The natural substrata of *T. inaequalis* and *T. curvatis* are not known, and although the spores of *T. aquatica* were collected from leaves in water it is possible that colonization of the leaves took place out of water. Preliminary experiments with *T. aquatica* spores (Webster, 1959) indicate that they have a low trapping efficiency in comparison with the common tetraradiate hyphomycete spores so common on submerged deciduous leaves.

Tricellula aquatica sp.nov.

Mycelium in cultura septatum, hyalinum ad 4 μ latum. Conidiophora simplicia aut composita. Conidia forma 'Y', tricellularia, 9–12 × 5–6 μ, cellula basalis inaequilateralis, aut cylindricalis aut clavata, 4·5–5 × 2·2–2·5 μ. Cellulae superiores duo, a cellula basali junctura arta (et nonnunquam muro) separatae sunt, obclavatae, angustae superne factae, 4–6 × 2–2·2 μ.

Hab. In foliis submersis putrescentibus *Alni glutinosae*, *Castaneae sativae* et *Hederae helicis* in flumine Porter prope Sheffield, Anglia. Specimen typicale Mycological Herbarium, University of Sheffield No. 2311 typus est.

Mycelium in culture septate, hyaline up to 4 μ wide. *Conidiophores* simple or compound. *Conidia* Y-shaped, 3-celled, 9–12 × 5–6 μ. Basal cell cylindrical, club-shaped, or inaequilateral, 4·5–5 × 2·2–2·5 μ. The two upper cells are separated from the basal cell by a constriction (and occasionally septa), obclavate, tapering upwards, 4–6 × 2–2·2 μ.

On submerged decaying leaves of *Alnus glutinosa*, *Castanea sativa* and *Hedera helix* in River Porter, near Sheffield. Cultures have been sent to the Commonwealth Mycological Institute, Kew, and the Centraalbureau voor Schimmelcultures, Baarn.

It is a pleasure to thank Prof. C. T. Ingold, Dr R. H. Haskins and Miss A. L. van Beverwijk for cultures and for helpful advice, and Mr A. O. Hulton for the Latin diagnosis. This work was aided by a grant from the University of Sheffield Research Fund.

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MYCORRHIZA IN THE GRAMINEAE

I. VESICULAR-ARBUSCULAR ENDOPHYTES, WITH SPECIAL REFERENCE TO THE EXTERNAL PHASE

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(With Plates 24 and 25 and 4 Text-figures)

Visual examination of roots of a range of grass species from different habitats and communities has shown that the main infecting organisms are Phycomycete endophytes of the vesicular-arbuscular type. An attempt was made to assess host-endophyte relations by examining concurrently all the fungal phases (internal mycelium, external mycelium and penetration). The internal mycelial phase in grasses corresponds to descriptions of similar endophytes in other plants. External mycelial development may be profuse in certain samples. The hyphae of this phase display dimorphism; there are thick-walled aseptate hyphae, and thin-walled hyphae which often become regularly septate. The former constitute the permanent basis of the external mycelium, the latter being mainly temporary.

External vesicles may be formed singly and are sometimes furnished with a rudimentary hyphal sheath. In certain samples of sand-dune grasses different types of vesicular aggregates have been noted. These are considered to correspond to fruit-bodies of the genus *Endogone*.

Root penetration takes place by hyphae entering either epidermal cells or root hairs. In certain samples, the numbers of hyphae penetrating through epidermal cells were as frequent as one per mm., while up to 92 % of the root hairs were penetrated directly.

From a consideration of host-endophyte relations, it was concluded that the fungi could possibly provide a mechanism for transfer of substances from soil to root. This could take place particularly during the early stages of the infection cycle in samples where abundant external mycelium and numerous root hair penetrations were present.

Other types of fungi also have been observed in grass roots. Among filamentous types, a *Rhizoctonia* and fungi with brown septate hyphae were present in many samples. Non-filamentous fungi such as *Olpidium brassicae* and *Ligniera junci* were frequently noted. It is considered that confusion with regard to vesicular-arbuscular mycorrhiza has been partially due to the dimorphic character of endophytes causing such infections. The frequent presence of other types of fungi in roots presents an additional confusing factor.

INTRODUCTION

Records of vesicular-arbuscular mycorrhiza in the Gramineae are extensive. However, most reports record presence or absence of endophytes in different species with little detail (Peyronel, 1922*a*, 1923–24; Mason, 1927; Dominik, 1951; Truszkowska, 1951) and often the host species have not been mentioned (Samuel, 1926; Asai, 1934; Neill, 1944). With more detailed observations, emphasis has been on the mycelium in the roots (McLennan, 1926; Simmonds & Ledingham, 1937; Winter, 1953; Strzemska, 1949, 1952, 1953; Tolle, 1958). Less attention has been paid to the mycelium of endophytic fungi occurring on the root surface and free in the soil outside the roots.

The structures characterizing this type of mycorrhiza have been regarded as functioning in the transference of food materials from fungus to higher plant. The vesicles were thought to function as storage organs, and the storage material was released into the host cells by digestion of arbuscules (Butler, 1939). Such conclusions, however, were based on examination of internal mycelium alone, with little reference to external mycelium. While there may be ultimate gain to the root in this way, it would consist only of material derived from host cells in the early stages of infection. It has been pointed out (McLuckie & Burges, 1932; Harley, 1950, 1952) that, when digestion of arbuscules is observed, it should not be assumed that this indicates absorption by the host of food materials originally obtained from the soil by the fungus. For such to occur 'requires hyphal connexions with the soil in significant quantity and an external mycelium absorbing soil substances' (Harley, 1950).

Since isolation of vesicular-arbuscular endophytes and experimental synthesis of the association have proved extremely difficult, little evidence has been available regarding the effect of endophytes on growth of the higher plant. However, recent work has had greater success in that direction (Mosse, 1957; Hawker & Ham, 1957; Peuss, 1958).

The work reported here represented an attempt to assess the host-endophyte relations of vesicular-arbuscular mycorrhiza in grasses by observing concurrently all phases of the endophytes. Greater emphasis has been placed on the external mycelial phase.

MATERIALS AND METHODS

Root samples of a range of grasses and cereals were collected from different sites throughout the country. The amount of infection and external mycelium varied greatly in different habitats and communities. Samples in which good infection and/or abundant external mycelium occurred were noted and used for more detailed observations. Some samples were used for observations on internal mycelium, some for external mycelial studies and some for both. Relevant data regarding the samples are given in Table 1. Numerous other samples have been examined for the presence of endophytic fungi.

Bulk samples of grass roots were obtained in the field by digging up one or more plants. With tussock-forming species such as *Dactylis glomerata*, a single clump of the grass gave an almost pure root sample. With creeping types of grasses or with a mixed grass flora, a turf was extracted and separated into its component species in the laboratory. With such samples great care was taken in the separation of the roots of different species. When pure samples were obtained, the root systems were cut from the foliage and fixed in formal acetic alcohol until further examination could be carried out. Excess washing was avoided, since this reduced the amount of mycelium on the root surfaces.

The identification of the fungi present in and on grass roots was facilitated and confusion reduced when whole roots rather than sectioned material were examined. With the small diameter and hyaline cortex of the fibrous roots of most species, the morphology of the fungi present can easily be observed by staining whole roots and examining them with strong transmitted light. The most successful stain was found to be cotton blue and Sudan IV in lactophenol. With whole stained roots, details of infections could be observed quickly and a complete picture of the distribution of the fungi in the root obtained. Further, external mycelium remained on the root surface, whereas with sectioned material only short segments were visible. Although most observations were made on whole unsectioned roots, sections were made of selected material and stained in Heidenhain's haematoxylin.

Table 1. *Root samples used in studies of vesicular-arbuscular endophytes*

			Used for studies on	
	Locality	Date of collection	Internal mycelium	External mycelium
Ammophila arenaria (L.) Link (marram grass). Field and potted material	Sand dunes: Gibraltar Point	June 1954	+	+
	Southport, Lancs.	May 1954	−	+
	St Cyrus, Kincardineshire	July 1951	−	+
Agropyron junceiforme (A. & D. Löve) A. & D. Löve (sand couch-grass)	Sand dunes: St Cyrus	July 1951	−	+
A. pungens (Pers.) Roem. and Schult (sea couch-grass)	Salt marsh: Gibraltar Point	September 1952	−	+
Arrhenatherum elatius (L.) J. & C. Presl. (oat-grass)	Sand dunes: St Cyrus	July 1951	−	+
Avena fatua L. (wild-oat)	Shetland Is.	September 1949	+	−
A. sativa L. (cultivated oat)	Shetland Is.	September 1949	+	−
	St Cyrus	July 1949	+	−
Dactylis glomerata L. (cock's-foot). Field and potted material	Aberdeen	October 1950	+	+
Festuca ovina L. (sheep's fescue)	Charnwood Forest, Leicestershire	July 1952	+	+
F. rubra L. var. *arenaria* (Osb.) Fr. (sand fescue)	Sand dunes: Gibraltar Point	September 1952	+	+
		August 1953	+	−
		June 1954	+	+
		July 1957	+	+

Samples collected in the field were reduced in bulk by bunching and cutting the roots into segments *c.* 0·5–1 cm. long. These were placed in a 1 in. layer of water in a rectangular dish, mixed thoroughly and spread out uniformly. The bulk quantity of segments was then successively halved until a convenient quantity remained for staining. A rapid indication of whether infection was present could generally be obtained by gently heating a few segments of the sample in a drop of cotton blue stain. For more extensive observations, the whole sample was immersed in a beaker of stain and heated until steaming. From the stained sample, segments were placed closely together in a rectangular area on a large slide (3 × 1 in.), mounted in lactophenol and covered with a $2\frac{1}{2} \times \frac{3}{4}$ in. coverslip. The preparation was ringed, labelled with appropriate details and stored. In one 'root slide' of this type there was available for rapid examination a large number (up to 300) of root segments drawn off at random from a bulk field sample.

Studies on the external mycelium and penetration phases were also made from root slides. In samples where extensive external mycelium occurred, clumps could be picked off the roots and stained to give permanent or semi-permanent preparations.

Vesicular-arbuscular endophytes

The internal mycelial phase

In morphology, the internal mycelium of grass endophytes corresponds closely to many previous descriptions of similar endophytes in grasses and other plants (McLennan, 1926; McLuckie & Burges, 1932; Lihnell, 1939; Neill, 1944). In typical vesicular-arbuscular infections in grass roots, the mycelium in the tissues develops from hyphae penetrating through epidermal cells or root hairs. The hyphae are often extremely coiled in the vicinity of the initial infection.* Septa in the internal hyphae are rare but sometimes isolated septa may be formed, cutting off empty from full segments. Both coiling and septation are more conspicuous in hyphae in the outer cortical cell layers and are generally absent in hyphae deeper in the tissues. With some infections the coiling stage may be absent, the penetrating hyphae then branching and passing directly into the inner cortical layers.

The first of the characteristic structures to be formed are the arbuscules. These are usually developed in the inner cortical cells. Each cell becomes occupied by an arbuscule and the inner layer shows as a densely-stained cylinder of cells surrounding the stele (Pl. 24, fig. 1). Often the conspicuous arbuscular layer may be absent, but with arbuscules scattered in other cell layers. In whole roots they show as dense granular masses filling the lumen of the occupied cell. However, in sections they appear as the densely tufted mass of branchlets which has been described by so many workers.

Following arbuscular development, the vesicles are formed mainly in the outer cortical cell layers. In general, these are located in an intracellular position and their size and shape often correspond to that of the occupied cell. Usually the vesicles remain in open connexion with the bearer hyphae but sometimes they become separated by a continuation of the vesicular wall, thereby giving a chlamydospore-like appearance. This has also been reported by Peyronel (1923) who maintained that many such vesicles developed into sporangia. This was never observed in grass roots,

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* Although such terms as 'infection', 'host', etc., are frequently used, this does not imply that the associations are considered to be pathogenic.

In grass roots, the main development of hyphae, arbuscules and vesicles is intracellular, with the latter two structures localized in layers. This would correspond to the infections placed by Gallaud (1905) in his *Paris quadrifolia* series. With infections in some species, and particularly in *Avena sativa*, hyphae and vesicles may be intercellular, corresponding more to Gallaud's *Arum maculatum* series. Endophytic hyphae have never been observed to invade the endodermis, nor the stele nor the actively growing root tip.

In many of the infections observed in grass roots, the internal mycelium passes through the stages previously reported (McLennan, 1926; McLuckie & Burges, 1932; Butler, 1939), with the removal of contents from hyphae and vesicles. These contents can be released into host cells via the arbuscular stage. However, further evidence is necessary before it could be stated that, as a general rule, 'the fungus is used by the root as a source of carbonaceous nutrient' (McLennan, 1926).

The external mycelial phase

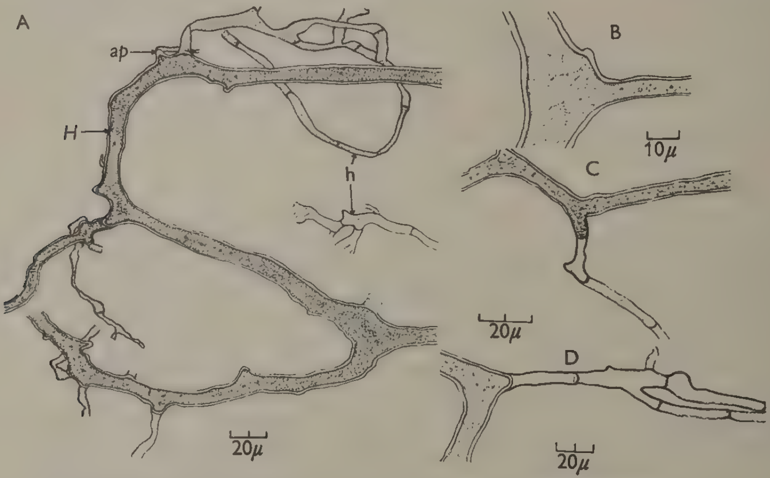
The amount of external mycelium of vesicular-arbuscular endophytes associated with grass roots varied greatly in samples from different habitats and soil types, and with different species. It was completely absent in some samples or was present in the form of a few isolated hyphal fragments. With other samples, the mycelium formed extensive wefts surrounding the roots and practically obscuring them.

Profuse external mycelium has been noted in many root samples from fixed sand dunes. The bulk of the mycelium appeared to be associated with living roots but frequently extensive clumps could be extracted from the sand—by flotation in a Petri dish of water—after the roots had been removed. These clumps grew round fragments of organic matter, which were often recognizable as old decaying roots. In addition to developing profusely over dead roots, the mycelium spread over other fragments of organic debris and over sand particles in the vicinity (Pl. 24, fig. 2). The profusion of development suggested that these fungi may be able to exist saprophytically on dead roots and other organic matter. Further evidence would be necessary, however, to confirm the supposition that the external mycelium can grow and develop in the absence of living roots.

A most remarkable morphological feature is the striking variation in diameter (from 2 to 27 μ) among hyphal filaments (Pl. 24, fig. 3), with accompanying variation in wall thickness. On this basis, two types of interconnected hyphae can be discerned as relatively distinct, though intermediate forms do occur. These will be referred to as the 'thick-walled' and 'thin-walled' hyphal elements.

The thick-walled element forms the permanent basis of complexes of external mycelium and consists of very stout, coarse hyphae. A characteristic and conspicuous feature is the presence of 'unilateral angular projections' (Butler, 1939) (Pl. 24, fig. 4; Text-fig. 1 A). The main trunk hyphae are 20–27 μ diameter and give rise, by subdichotomous branching, to finer thick-walled hyphae of 7·5–10 μ diameter. The walls are irregularly thickened and may be as much as 3 μ thick where angular projections occur. They separate into two layers with applied pressure (Pl 24, fig. 5).

The thick-walled hyphae are almost always filled with dense cytoplasmic contents. Oil globules are a conspicuous feature when Sudan IV is used in staining. The thick-walled hyphae are typically non-septate, although isolated septa may occur. Septa are more usually associated with vesicles, but may also be found between a full and an empty segment or between a fine branch and its parent hypha. The hyphae of the thick-walled element are multinucleate (Pl. 24, fig. 5), though nuclei are not always distributed evenly along lengths of hyphae. Congregation of nuclei occurs in certain segments, especially where branching takes place. Some lengths of hyphae are devoid of nuclei.



Text-fig. 1. Thick-walled and thin-walled hyphal elements. From mycelium on the roots of *Dactylis glomerata*. A, hypha of the thick-walled element with many angular projections and thin-walled hyphae attached; *H*, thick-walled hypha; *h*, thin-walled hypha; *ap*, angular projection. B, thin-walled hypha in direct connexion with thick-walled hypha. C and D, septation in thin-walled hyphae. (Camera lucida drawings.)

In contrast to the permanent thick-walled hyphae, the majority of the thin-walled hyphae are temporary and ephemeral in nature and, although non-septate in the early stages, many typically become septate. The hyphae of the thin-walled element are smooth with few irregularities in outline, and are 2–7 μ in diameter (Pl. 24, fig. 3). They appear hyaline, which may be due to the absence of contents. Development of thin-walled hyphae takes place in two main ways; first, by direct development as lateral branches from thick-walled hyphae (Text-fig. 1) and, secondly, by repeated branching of thick-walled hyphae giving progressively finer filaments with progressively thinner walls.

Thin-walled hyphae arising as laterals show a progression of stages with regard to septation. First, some are non-septate with direct protoplasmic connexion to the parent hypha of the thick-walled element (Text-fig. 1 B). Secondly, filaments which have become regularly septate may not be completely cut off, the first septum being beyond the junction of the two types of hypha (Text-fig. 1 C). Thirdly, the fine hyphae may be regularly septate and cut off from the bearer hypha at the junction (Text-fig. 1 D). Finally, shrivelled fragments remain attached to thick-walled hyphae (Text-fig. 1 A). It would appear that the lateral thin-walled hyphae grow directly out from the hyphae of the permanent element and for a time remain in direct protoplasmic connexion with it. Later, the contents of the thin-walled hyphae disappear, and protoplasmic parts become cut off from the empty parts by septa. Finally, when the contents of the thin-walled hyphae have more or less disappeared, a curved cross-wall occludes the common opening. The thin-walled hyphae disintegrate, leaving the conspicuous angular projections on the thick-walled hyphae as their former sites of attachment.

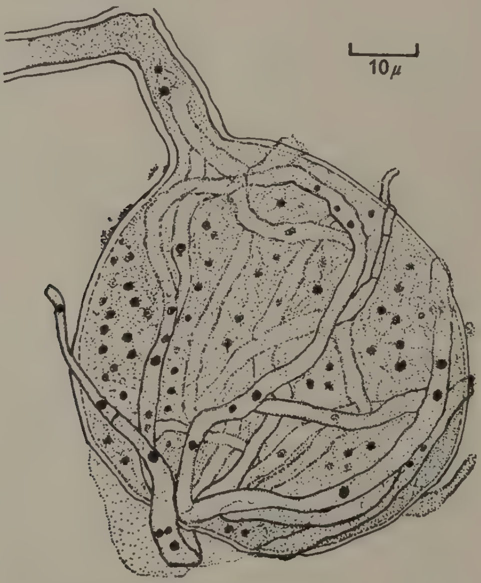
While septation is conspicuous in laterally borne thin-walled hyphae, it is not so evident in those which have arisen by repeated branching of thick-walled hyphae. Many of these hyphae will no doubt, with increase in size and thickening of walls, develop into thick-walled hyphae of future mycelial complexes, but it is doubtful whether any laterally borne hyphae act in this way.

The proportion of viable non-septate hyphae to hyphae which have lost their contents, becoming septate and disintegrated, varied with different root samples. Presumably the degree of septation would depend on the activity of the mycelium at the time of collection of the sample. Absence of septa in viable thin-walled hyphae would indicate that the mycelium was actively growing at the time of collection. On the other hand, a large number of septate or shrivelled hyphae would indicate that the mycelium was nearing the end of an active period of growth.

External vesicles and vesicular aggregates

Terminal, chlamydospore-like swellings or spores which are formed on external mycelium of vesicular-arbuscular endophytes have generally been regarded as analogous to vesicles formed in root tissues and are commonly referred to as 'external vesicles' (Butler, 1939). Such structures when formed on mycelium associated with grass root infections are generally borne terminally on subsidiary, thick-walled branches of the permanent element (Pl. 24, fig. 6). There is great variation in size and shape of vesicles, and dimensions of up to 150 μ or as low as 20 μ were noted. The vesicles, like the hyphae on which they are borne, are thick walled. The wall consists of two layers; a thin, rough, outer layer and a smooth, thicker, inner layer, which become obvious on crushing. The contents of vesicles are usually dense and contain oil globules and many nuclei. Most vesicles remain in open connexion with the bearer hypha (Text-fig. 2). Frequently, however, the vesicle with a small length of bearer hypha is cut off from the rest of the mycelial complex by a septum.

In both types of vesicles (those cut off and those not), hyphae are often observed in association with the vesicular surface (Text-fig. 2). It is sometimes difficult to ascertain the true disposition of these hyphae in relation to the walls of the vesicle. Sometimes the hyphae appeared to be located inside the vesicles and may have belonged to parasitic fungi, but more often they appeared to be present on the surface and arising from the outer wall. It is unlikely that these hyphae function as germ tubes, since they are not densely cytoplasmic nor do they grow out from a germinating body. As they are mostly empty and closely adpressed to the vesicular surface, it is believed that they represent a rudimentary protective sheath.



Text-fig. 2. External vesicle with fine hyphae on the surface. (Camera lucida drawing.)

In addition to isolated vesicles borne singly on external mycelium, more complex structures consisting of aggregations of vesicles have been found. These have been noted on external mycelium from roots of *Ammophila arenaria* and *Festuca rubra* var. *arenaria* from the fixed zones of sand dunes. They resemble fruiting bodies of certain species of the genus *Endogone* Link. Members of this genus have been recorded as forming vesicular-arbuscular mycorrhiza (Peyronel, 1924, 1937; Mosse, 1953, 1956).

Vesicular aggregates found in association with grass mycorrhiza vary greatly in size but those noted so far have always been less than 0·8 mm. In colour they show various shades of brown. They also vary greatly in number and size of contained vesicles, and in the composition of a background matrix. In their simplest form, the aggregates consist of a few vesicles originating from a single branched hypha (Pl. 25, fig. 7). As many as forty vesicles originating from numerous hyphae may become aggregated in this way to form a fairly compact mass (Pl. 25, fig. 8). With some aggregates, intertwined hyphae are present between the vesicles, often forming a dense matrix and giving the structure a more compact appearance. More frequently, vesicles may be embedded in a background matrix consisting of varying amounts of organic matter and hyphae, or the matrix may consist of organic material with few hyphae. After careful washing of certain sand-dune samples, many particles of organic matter remained attached to the external mycelium or to the root surfaces, and when crushed many were found to contain embedded vesicles (Pl. 25, fig. 9). Such particles had no distinguishing external appearance and only when they were crushed could the embedded vesicles be seen. The enveloping organic matter was rarely recognizable as root material.

The vesicular aggregates found associated with grass mycorrhiza, whether formed in a coherent cluster or embedded in various amounts of hyphae or organic matter, were in no way as regular or uniform in structure as the sporocarps of the *Endogone* sp. found to form vesicular-arbuscular mycorrhiza with fruit and other plants (Mosse, 1956). Moreover, the vesicles in the aggregates did not vary markedly from those formed in isolation on external mycelium. They did not become separated from bearer hyphae as had been noted for spores in the *Endogone* sp. sporocarps investigated by Mosse (1956). Individual vesicles were always borne on a single parent hypha. They were generally spherical or pear-shaped but great irregularity in shape has been noted. The size varied greatly, with dimensions between 25 and 75 μ.

Vesicles, formed either in isolation or in aggregates, are the only type of spores which have been found in association with external mycelium on grass roots. Large soil-borne spores and echinulate spores reported on mycelium associated with vesicular-arbuscular mycorrhiza in clover (Gerdemann, 1955) and barley (Ohms, 1956) have not been seen.

The penetration phase

When vesicular-arbuscular endophytes enter grass roots, initial penetration can take place either by way of a root hair, by a cell of the piliferous layer or by an exodermal cell in older roots where the piliferous layer has been sloughed off. In any one sample, all root infections result predominantly from either root hair or epidermal (piliferous layer) cell penetrations. In penetration of an epidermal cell, a hypha comes into contact with the root surface and forms an appressorial-like body (Pl. 25, fig. 10). These structures are also associated with penetration of root hairs but not so regularly nor conspicuously. From the swelling on the root surface, a penetration hypha enters the underlying cell. It is believed that these infection swellings resemble true appressoria of parasitic fungi of the higher groups (Brown, 1936), and that this indicates that entry is by mechanical means. Both thick-walled and thin-walled hyphae are capable of root infection, and both types of hypha can infect through root hairs or epidermal cells. If a thick-walled hypha penetrates, the diameter decreases and the walls become smooth and thin as the hypha grows into the root.

The numbers of penetrating hyphae vary greatly in different samples, but accurate estimates are difficult to obtain. With root-hair infections, the hyphae are generally broken off at the actual point of penetration. With epidermal cell penetrations, it is much easier to preserve connexions between internal and external mycelium (Pl. 25, fig. 11). Numbers of penetrating hyphae found in samples of sand-dune grasses are shown in Table 2. Where hyphae penetrate epidermal cells, a small number may be responsible for extensive lengths of root infection (Table 2 *a*). When penetration is by root hairs, hyphae are much more numerous. In heavily infected samples, each root hair of certain root segments may show active or disintegrating hyphae (Table 2 *b*).

Table 2. *Numbers of penetrating hyphae*

(*a*) Through epidermal cells: counts in *A. arenaria* from fixed sand dunes

Segment no.	Length of infection (mm.)	penetrating hyphae
1	0·85	1
2	1·70	2
3	2·36	3
4	2·50	2
5	4·25	3
6	4·25	6
7	5·10	4
8	2·40	2
Totals	23·41	23

(*b*) Through root hairs: counts from a typical segment (4·5 mm. long) of *F. rubra* var. *arenaria* from fixed sand dunes

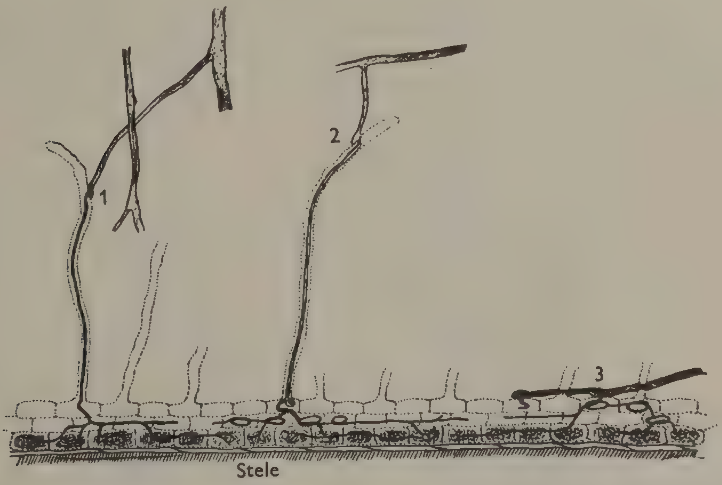
	Total no. root hairs	No. root hairs with	
		Viable hyphae	Hyphal remains
Number	131	6	115
Percentage	—	4·6	87·8
		92 %	

Interrelationships between external mycelium, internal mycelium and host

The aim here was to observe the relationships between external and internal mycelium, and to ascertain whether a mechanism for the transfer of nutrients from the soil to the root via the fungus could exist. Samples of grass roots from fixed sand dunes which showed strong development of external mycelium and a high degree of root infection were particularly useful in furnishing information on these features. Many penetrating hyphae could be traced directly to extensive mycelial complexes around the root and there is no doubt that the internal mycelium is often connected to viable mycelium in the soil.

During infection three main stages can be recognized. The first stage includes the initial infection and subsequent spread of hyphae longitudinally in the cortex. Extensive coiling may occur in the outer cortical cells. During this stage the internal mycelium is directly connected to the external mycelium by viable hyphae and is probably nourished by materials transported from the external mycelium.

The second stage is seen when hyphae penetrate to the inner cortical layer of cells and the arbuscules are formed in a zone around the stele (Text-fig. 3; Pl. 24, fig. 1). The penetrating hyphae then begin to disintegrate and the thin-walled hyphae become septate as their contents disappear (Infection 2, Text-fig. 3). Penetrating hyphae of the thick-walled type still maintain protoplasmic connexion between the two mycelial phases (Infections 1 and 3) and provide channels along which materials could be transported between them.



Text-fig. 3. Diagrammatic representation of the relationships between external mycelium, penetration and internal mycelium during the arbuscular stage. Only fragments of external hyphae are shown. Three types of infection are represented: 1, by a thick-walled hypha through a root hair; 2, by a thin-walled hypha which has become empty and septate; 3, by a thick-walled hypha through epidermal cells.

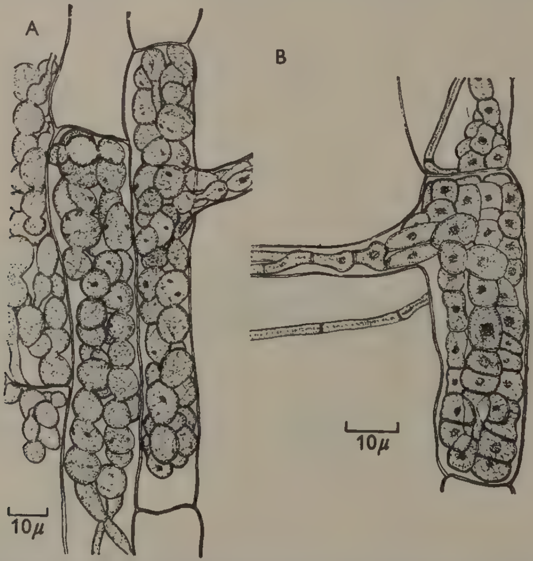
In the final phase, vesicles form in the outer cortical layers. By this time the majority of the penetrating hyphae have disintegrated and connexion between internal and external mycelium is lost. Together with hyphae in the outer cortical layers, the penetrating hyphae become empty and septate or completely disintegrate. When this final stage is reached, there is little likelihood of any significant amount of food materials passing between external and internal mycelium. Further development of the fungus in the root would then be independent of the external mycelium, and must occur either at the expense of materials already synthesized or of materials absorbed from host tissue.

In any one field sample of grass roots, the majority of infected roots show the same stage of endophyte development, though some overlapping of stages does occur. The particular stage of development could not be related to any feature pertaining to the sample, e.g. time of sampling, weather or soil conditions, etc. The cycle of infection appears to take place repeatedly over the growing season while new roots are being formed.

Other fungi present in grass roots

In addition to vesicular-arbuscular endophytes many other fungi of different types are common in and on grass roots. These usually cannot be identified by direct observation as only hyphae are present. Some types of hyphae occur sporadically and some are consistently present. Frequently more than one type of fungus may be present in the same root segment. The fungi observed consist of two main groups: filamentous types, generally with septate hyphae, and non-filamentous forms belonging to the Chytridiales and Plasmodiophorales.

The main filamentous forms observed were represented by a Rhizoctonia-like type of endophyte similar to those found in orchids, and various fungi with mycelia of brown septate hyphae. The appearance of the Rhizoctonia-like fungus corresponded closely to similar infections figured by Peyronel (1923–24), Truscott (1934) and Hildebrand & Koch (1936) in other plants. The conspicuous feature is the formation of systems of beaded cells or oidia (Text-fig. 4A). The oidia are regularly rounded or oval with dimensions of 6–10 μ. The associated septate hyphae are very fine with a diameter of 1–2 μ. Hyphae and oidia showed a homogeneous granular appearance when stained with cotton blue, with the binucleate condition of the oidia often recognizable (Text-fig. 4A). Hyphal coils or peletons of the type seen in orchids were never observed in grass roots.



Text-fig. 4. In epidermal cells of *Dactylis glomerata*. A, oidial systems of a Rhizoctonia. B, hyphae and oidial systems of a brown septate fungus. In both cases entry has taken place through root hairs, the bases of which are shown. (Camera lucida drawings.)

Rhizoctonia-like infections were always restricted in extent. Usually only three or four adjoining cells contained hyphae and oidia, though lines of cells up to 2 mm. were occupied by the fungus. The infections were always confined to the superficial cortical cells, and caused no apparent injury. Fungi of this type have been noted in the roots of many grasses from different habitats, but only a few segments in any one sample were infected. Profuse development as reported by Peyronel (1923–24) was never observed.

In almost every root sample examined, a fungal mycelium of brown septate hyphae was observed. These hyphae ran over the root surface and penetrated the root to form brown bead systems in the outer cortical cells. Usually, the bead systems did not occupy more than one cell at any one penetration point (Text-fig. 4B). The consistency in hyphal size (2·5–4 μ), and in the appearance of the beaded cells, in the various root samples suggests that these fungi are taxonomically related, though they could not be identified by visual means alone. The beads were oval or oblong and measured up to 14 μ. Brown septate mycelium was associated with all kinds of roots, but was present more profusely on roots showing disintegration and decay. In some samples, mycelium of this type was observed on over half the roots placed on the root slides.

The most frequent Chytridiaceous fungus is represented by *Olpidium brassicae* (Wor.) Dang. which has been frequently reported in roots of various plants (Sampson, 1939). In grass roots, both resting sporangia of the stellate form (Pl. 25, fig. 12) and variously shaped zoosporangia, frequently with exit tubes, have been observed.

Plasmodiophoraceous species are common in certain samples. Most frequently the spores are in the form of compact clusters with no characteristic shape which occupy cortical cells and appear to belong to *Ligniera junci* (Schwartz) Maire & Tison (Cook, 1926). Only very rarely have the hollow spore balls of *Sorosphaera radicale* (Cook & Schwartz, 1929) been observed in swollen tips of root hairs (Pl. 25, fig. 13). Zoosporangial and naked plasmodial stages of these fungi have also been noted.

Although these non-filamentous fungi are regarded as occurring most frequently in wet soils, they are often present in grass roots from dry habitats. Over 50% root infection by *Olpidium brassicae* has been noted in some samples from sand dunes. *Ligniera junci* was also frequent in samples from sand dunes. Neither of these fungi caused any apparent damage to host roots.

DISCUSSION

In few relationships between fungi and higher plants has there been so much confusion as with mycorrhiza of the so-called vesicular-arbuscular type. This has been discussed by Harley (1950). Confusion in early work arose largely because endophytes which formed vesicles and arbuscules were regarded as being similar to those forming mycorrhizas of different appearance in such plants as the orchids and certain heath families. The situation was partially clarified by Peyronel (1922*b*, 1923, 1923–24), who showed that the vesicles and arbuscules described by previous authors (Janse, 1896; Gallaud, 1905) were formed by a phycomycete. A second endophyte resembling the Rhizoctonias of orchids was also present in plants infected by the phycomycete. Both types of endophyte were of widespread distribution but the phycomycete occurred more frequently and was of greater importance. While the work of certain subsequent workers supported Peyronel's findings of 'double infection' (McLuckie & Burges, 1932; Hildebrand, 1934; Koch, 1935; Truscott, 1934; Hildebrand & Koch, 1936), there was still much confusion regarding the endophytes causing vesicular-arbuscular mycorrhiza (Harley, 1950).

Vesicular-arbuscular mycorrhiza in grasses appears to be caused by a single type of endophyte, typically with non-septate hyphae. Marked dimorphism is displayed between internal and external mycelium, and there is frequent development of regular septation in certain external hyphae and in hyphae in outer cortical cells. These features have been noted in similar endophytes of many other plants (McLuckie & Burges, 1932; Koch, 1935; Sievers, 1958; Mosse, 1959) and are no doubt responsible for much confusion in this type of mycorrhiza. An additional confusing factor when studying vesicular-arbuscular endophytes is that structures of many other types of fungi may also be present in the roots. Indeed, grass roots may provide habitats for fungal successions with the endophytes as primary colonizers (Waid, 1957).

Peyronel's (1924, 1937) observations that phycomycete endophytes of vesicular-arbuscular mycorrhiza could belong to the family Endogonaceae were substantiated by Mosse (1953, 1956). Studies with grass roots provided further evidence that these infections can be induced by Endogone-type fungi (Nicolson, 1958). The vesicular aggregates described resemble the fruiting bodies of certain species of the genus *Endogone*. There is little doubt that these structures are fruiting bodies, with the vesicles corresponding to the chlamydospores which are an important spore form of this genus. No other type of spore has been noted in these aggregates, hence they would appear to belong to chlamydosporic species (Godfrey, 1957). The lack of uniformity, particularly with regard to appearance and composition of background matrix, makes identification difficult. Certain specimens observed have affinities with *Endogone fasciculata* Thaxter and *E. vesiculifera* Thaxter. Both these species form variably shaped, loosely coherent spore masses with varying amounts of organic matter incorporated in the background matrix (Thaxter, 1922). However, nothing corresponding to the zygospores of the former or to the thin-walled clavate vesicles characteristic of the latter species was observed. Further study would be necessary to reveal the true identity of the vesicular aggregates on grass roots.

Although the evidence referred to above indicates that vesicular-arbuscular infections are caused by *Endogone* spp., it has been shown that other phycomycete fungi can cause similar infections (Hawker, Harrison, Nicholls & Ham, 1957; Hawker & Ham, 1957). These workers have shown that *Pythium ultimum* forms vesicular-arbuscular mycorrhizas with certain plants, particularly the Liliiflorae. For these associations, no external mycelial phase has been described.

Sufficient evidence is not yet available to indicate whether the mycorrhizas caused either by *Endogone* or by *Pythium* spp. are universally distributed in nature, or whether any factors related to host or habitat determine which type of endophyte may be present. In spite of the vast amount of work which has been done, much more is required before a clearer picture of vesicular-arbuscular mycorrhizas and the endophytes involved can be obtained.

In considering possible conduction of materials by the mycorrhiza in grasses, the endophyte has been regarded as a three-phase system consisting of internal mycelium, external mycelium and connecting hyphae. Two main considerations are involved; first, the numbers of connecting hyphae, and secondly, the length of time that the internal and external mycelium are actively connected by these hyphae. While it is difficult to obtain accurate counts, in certain samples of grass roots the numbers of penetrating hyphae were as high as 1 per mm. with epidermal cell penetrations and up to 92 % with root hair infections. These numbers, particularly with root hair entry, are considered sufficient to allow some food transference from external to internal mycelium, or in the reverse direction. In cereals, Winter (1953) has reported a similar number with epidermal cell entries (maximum of 10–20 per cm. length of root) but less than 3 % with root hair infections. However, on the basis of these results, he concluded that there was no evidence that hyphal connexions were sufficient to play any part in food transference from soil to root. Harley (1950), on the other hand, suggests that in some cases there may be sufficient connexions for food transference. He bases this suggestion mainly on counts made by McLuckie & Burges (1932) and by Lihnell (1939). Recently Mosse, (1959) has reported extensive epidermal cell penetration in fruit plants. With strawberries, entry points were as numerous as 21·1 per mm.

Regarding the time that internal mycelium and external mycelium may be directly connected by active penetrating hyphae, it has been found that such connexion may be maintained during the early stages of the infection cycle. This included the period of active penetration prior to formation of any of the characteristic structures and, to some degree, during the period of arbuscular formation. Thereafter, as the internal vesicles are formed, the numbers of penetration hyphae are gradually reduced, until eventually all connexion between the fungal phases is lost. Further information is required on the duration of these developmental phases, and on their relation to seasonal root development of the grass hosts.

It would appear, then, that vesicular-arbuscular mycorrhiza in grasses may provide a physical channel for food transference and absorption. However, this could equally well function to transfer materials from mycelium in the root to the external mycelium. Physiological studies would be required to indicate in which direction any such transfer might take place. Conclusive evidence of increased absorption is available for certain forms of ectotrophic mycorrhiza (Harley, 1956), but is lacking for vesicular-arbuscular mycorrhiza. In grass roots, transference is only likely to occur during the early infection stages where abundant external mycelium and numerous penetrations are present in the sample. So far, these requisite conditions have been noted with certain samples of sand-dune grasses, but whether they occur widely can only be determined by extensive examination of samples from different habitats and communities.

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EXPLANATION OF PLATES 24 AND 25

Plate 24

Fig. 1. Comparison between an infected and a non-infected root of *Festuca rubra* var. *arenaria*. The dark zone in the upper root is the arbuscular layer surrounding the stele. (× 75.)

Fig. 2. Profuse external mycelium developing over decaying root segments, fragments of organic debris and sand particles. Sample from fixed sand dunes, Gibraltar point. (× 38.)

Fig. 3. Showing variation in size and wall thickness between thick-walled and thin-walled hyphae. *H*, thick-walled hypha; *h*, thin-walled hypha. On *Ammophila arenaria* roots. (× 320.)

Fig. 4. Main trunk branches of the thick-walled element (*H*) bearing fine hyphae (*h*) and vesicles (*v*). Angular projections (*ap*) can be seen. On *Dactylis glomerata* roots. (× 75.)

Fig. 5. Nuclei in a hypha of the thick-walled element. Separation of the wall layers (*s*) can be seen in the side branch. On *D. glomerata* roots. (× 620.)

Figs. 6–9. External vesicles and vesicular aggregates on roots of *F. rubra* var. *arenaria*.

Fig. 6. Single vesicles on roots. (× 75.)

PLATE 25

Fig. 7. Small vesicular aggregate with six vesicles of varying sizes arising from a single hypha. (× 320.)

Fig. 8. Vesicular aggregate where the vesicles are not embedded in any significant amount of hyphae or organic matter. (× 90.)

Fig. 9. Vesicular aggregate where the vesicles are embedded in a particle of organic matter—connected to external mycelial complex (*m*) and a decayed root segment (*r*). (× 60.)

Fig. 10. External hyphae on root surface penetrating epidermal cells and forming appressorial-like swelling (*a*). *D. glomerata*. (× 350.)

Fig. 11. Epidermal cell penetration where two vesicles are connected to a side branch of the penetrating hypha. (Numerous oil globules are present.) (× 100.)

Fig. 12. Stellate resting sporangia of *Olpidium brassicae* in an epidermal cell. *Agropyron pungens*. (× 350.)

Fig. 13. Hollow spore balls of *Sorosphaera radicalis*. In swollen root hairs of *Cynosurus cristatus*. (× 450.)

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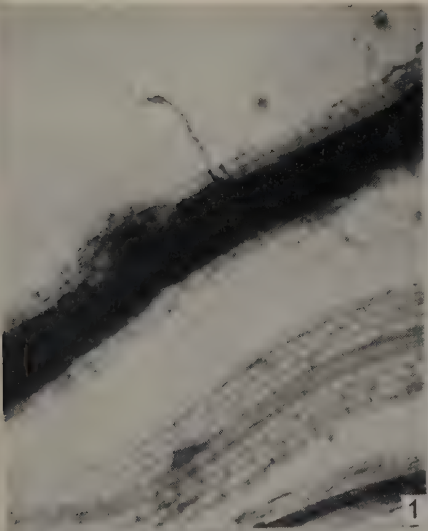
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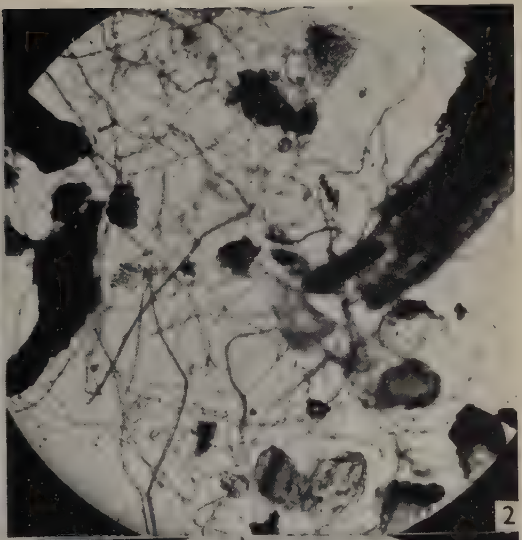
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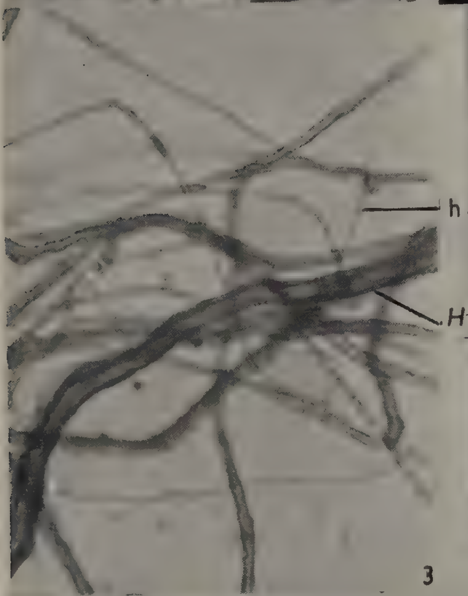

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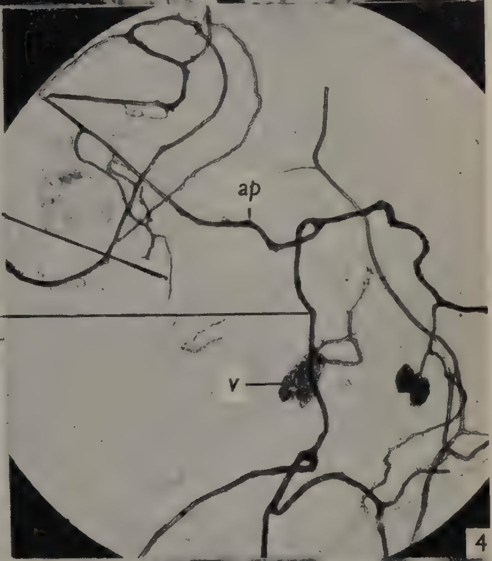

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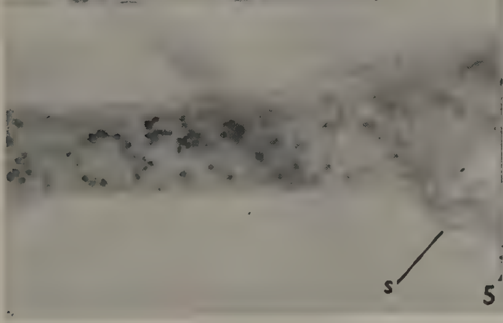

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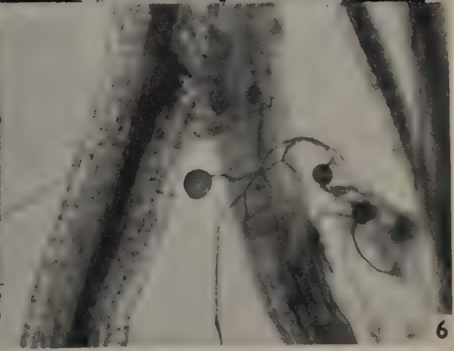
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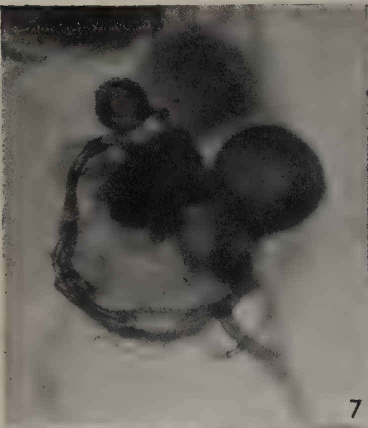

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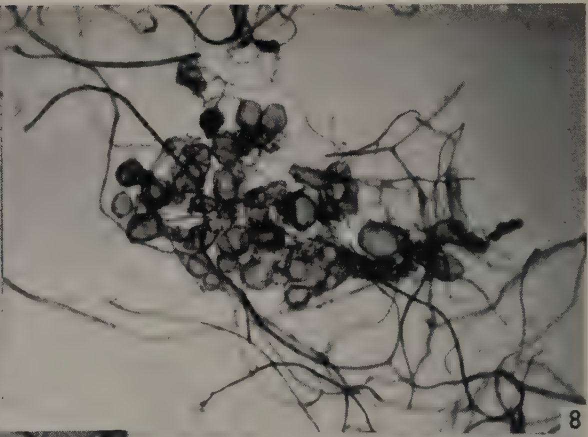

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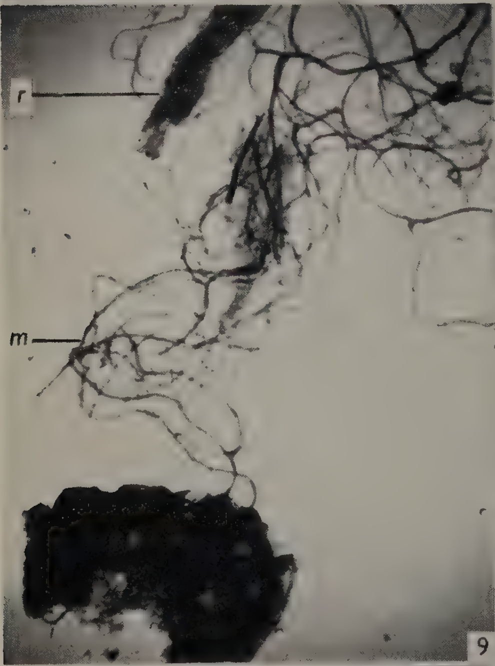

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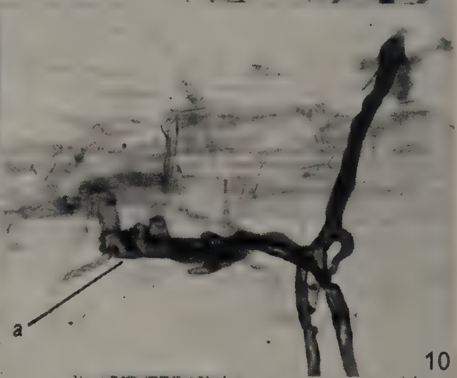

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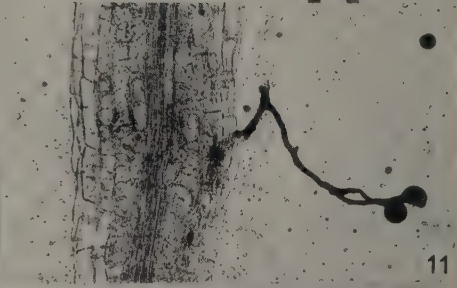

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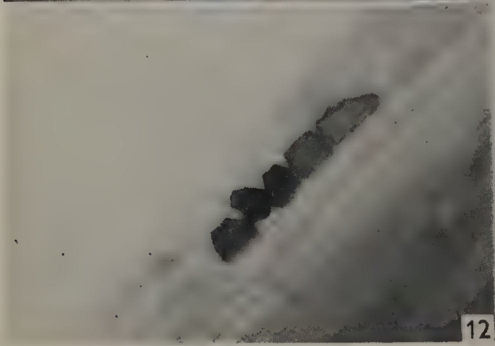

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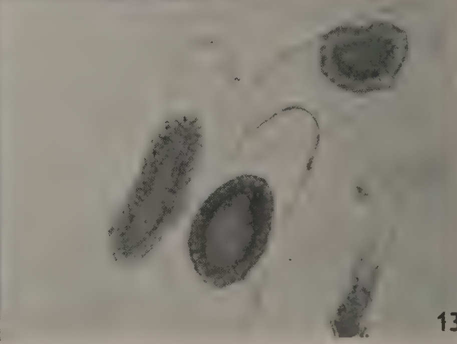

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Trans. Brit. mycol. Soc. **42** (4), 439–448 (1959).

OBSERVATIONS ON THE EXTRA-MATRICAL MYCELIUM OF A VESICULAR-ARBUSCULAR ENDOPHYTE

By BARBARA MOSSE

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(With Plates 26 and 27 and 3 Text-figures)

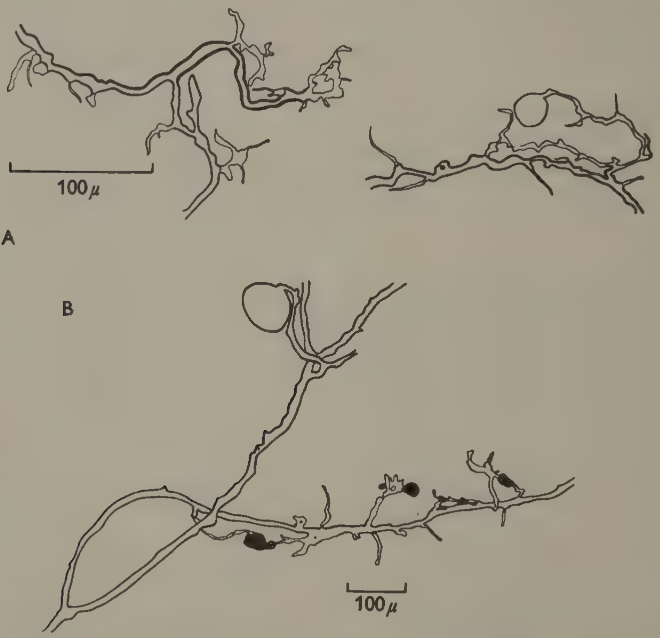
Information is presented about the morphology of the extra-matrical mycelium and the extent of its connexion with a vesicular-arbuscular endophyte in mycorrhizal roots. The number of hyphal connexions varied from 2·6 to 21·1 per mm. root length according to the season, environment and manurial treatment. The possible interpretation of these results is discussed.

Much thought has been given to the intimate co-existence of soil fungi and plant roots which finds its ultimate expression in the widespread phenomenon of vesicular-arbuscular mycorrhiza. The chief problems arising from such association can be summarized as follows: (1) Studies of the causal organism: (*a*) its identity, (*b*) its growth requirements. (2) Relationship of the fungus with its host: (*a*) its effect on root metabolism and plant growth, (*b*) its saprophytic activity in the soil. (3) Relationship of the fungus with other rhizosphere organisms.

Harley (1950), reviewing the information available on the soil phase of the vesicular-arbuscular endophyte, pointed out the need for further evidence concerning the frequency of hyphal connexion between mycorrhiza and the soil, and for further information on the ecology of the extra-matrical mycelium. When therefore an investigation was begun to study: (1) seasonal variation and distribution of mycorrhizal roots in the apple; (2) effect of different soil treatments on the mycorrhizal infection of strawberries grown in pots (Mosse, 1954), particular attention was paid to these points. The information obtained is considered under two headings: (1) a description of the extra-matrical mycelium; (2) the frequency of hyphal connexions, its relationship to seasonal and cultural factors, and circumstantial evidence concerning the saprophytic existence of this fungus in the rhizosphere.

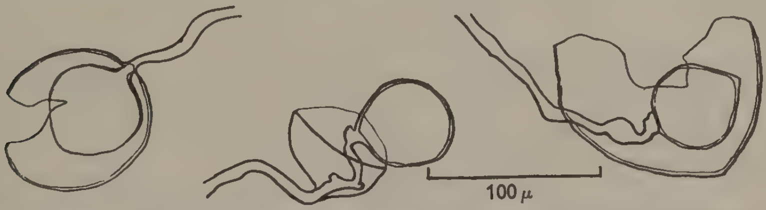
THE EXTRA-MATRICAL MYCELIUM

Three-year-old trees of the apple rootstock Malling II, grown in a nursery row in the field, were used for a monthly survey of mycorrhizal infection. If root samples were carefully collected in intact soil blocks and then gently soaked out in water, they frequently remained surrounded by a loose weft of translucent coarse hyphae, extending up to 1 cm. from the root surface. This hyphal weft (Pl. 26, fig. 1) was rendered more conspicuous by the fine particles of soil and organic matter which firmly adhered to it, even after gentle tapping, when unattached soil particles would break up and fall away. A high proportion (75 % or more) of the hyphal weft consisted of coarse, generally aseptate hyphae (up to 20 μ in width) with the highly characteristic unilateral angular projections and thick yellowish walls (up to 4 μ thick) that have frequently been described as distinguishing features of the extra-matrical mycelium of the endophyte (Butler, 1939; Peyronel, 1923–4; McLuckie & Burges, 1932). Such hyphae can often be traced back into the roots without great difficulty, and their continuity with the vesicular-arbuscular endophyte can be established with certainty.



Text-fig. 1. Tracing of the extra-matrical mycelium. A, taken from the root surface; B, soaked out of the soil. Note the haustoria-like branches firmly attached to soil particles.

In addition to the main network of permanent hyphae which at maturity are characteristically thick-walled, there is a system of thin-walled, usually short and often septate hyphae which appear to be more temporary structures (Pl. 26, fig. 2). They arise as lateral branches, often from the angular projections of already fully mature hyphae. Unlike the main hyphae which characteristically branch at an angle of 30–60°, the thin-walled secondary branches arise much more abruptly, usually at right angles to the parent hypha. They are very variable in shape, following the outline of root surfaces or of soil particles, to which they become firmly attached. Some haustoria-like branches of this type are shown in Text-fig. 1. Their outlines suggest that they may act as absorbing organs. If they fail to find a suitable attachment, they frequently become long and ribbon-like. Although there is a strong tendency to dimorphism, as above described, this is not rigid, and intermediate types of hyphae undoubtedly occur. Nicolson (1955, 1959) observed a similar tendency to dimorphism in the extra-matrical mycelium associated with the mycorrhizal roots of some grasses. Also extremely characteristic of the extra-matrical mycelium are the vesicle-like, round to pear-shaped spores that are nearly always borne terminally, either in groups at the end of a repeatedly branching main hypha, or on short lateral branches. The spores vary from 20 to 150 μ in diameter, are multinucleate with a dense, granular, deeply staining protoplasm, and have open connexions with the parent hypha when young. With age they become vacuolated and gradually develop a second spore wall internally. At first this is pale yellow, but it may darken later. Occasionally a basal plug develops across the spore neck, but more usually open connexion with the parent hypha is maintained. There is no evidence that these spores ever become detached from the extra-matrical mycelium by any regular mechanism of spore dispersal but, like the thick-walled coarse hyphae themselves, they can give rise to new hyphae and thus act as relatively resistant propagules after periods of drought. Proliferating vesicles (Text-fig. 2), like those attributed by Butler (1939) to fossil specimens of *Rhizophagus*, have also been found occasionally.



Text-fig. 2. Tracing of proliferating vesicle-like spores attached to the extra-matrical mycelium associated with mycorrhizal apple roots.

An extensive extra-matrical mycelium was regularly associated with the mycorrhizal roots of a range of fruit plants, e.g. raspberries, pears, plums, quinces and strawberries grown under orchard conditions at East Malling. It was especially well developed where the surface soil had been left undisturbed, as for instance under a straw mulch. The extra-matrical mycelium was even more extensive around apple and strawberry roots grown in pots in autoclaved soil, inoculated with *Endogone* sporocarps.

THE FREQUENCY OF HYPHAL CONNEXION

The frequency of hyphal connexion between mycorrhizal roots and the extra-matrical mycelium was measured by counting the number of entry points seen in serial longitudinal sections (30 μ thick) of infected rootlets cut on a freezing microtome. Fresh roots were used, and all the sections from each root were mounted and stained in a plastic mounting medium, polyvinyl lactophenol (Metcalfe & Richardson, 1949) to which a little cotton blue had been added. This produced preparations which remained clear and well stained for several years. All the sections were examined microscopically and an entry point was recorded for every hypha that crossed the outer cell wall of the epidermis. The possibility that some of the 'entry' points so recorded might in fact be points of egress was considered, but on the basis of anatomical evidence discussed below this does not appear very likely.

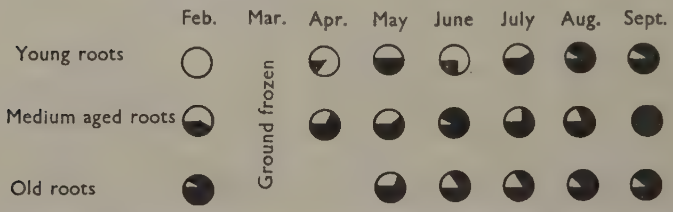
Entry into the root is usually preceded by the swelling and enlargement of the penetrating hyphal tip and the formation of a prominent appressorium on the root surface (Pl. 26, fig. 3). Often several infecting branches may arise from one appressorium. Therefore not every point recorded necessarily represents a separate connecting link between the endophyte and the extra-matrical mycelium. In addition, hyphae often travel along the root surface, giving rise to a series of infecting branches at intervals (Pl. 27, fig. 4). Both these factors tend towards a high frequency of recorded entry points in serial sections as compared to root squashes or whole mounts. On the other hand, the likelihood of including in the record other root-infecting fungi, a danger that was pointed out by Harley (1950), is considerably smaller in sections, where the character of the invading hyphae can be seen more clearly.

Entry points were recorded in three types of material: (1) apple roots collected in the orchard at three different times of the year. (2) strawberry roots infected naturally and by inoculation with an *Endogone* sp.; (3) clonal strawberry roots grown in a glasshouse in different composts.

Table 1. *Seasonal variation in number of entry points in apple roots*

	June	September	November
Entry points/mm. root length (mean of 10 roots)	4·6	9·7	10·7

Sig. diff. ($P = 0·01$) 2·3



Text-fig. 3. Proportion of mycorrhizal roots at different sampling dates.

The significantly smaller number of entry points in June (Table 1) coincides with a smaller proportion of infected roots at that time of the year. This is shown by the results of a study of seasonal variation in intensity of mycorrhizal infection (Text-fig. 3). Roots were divided into three age-groups according to their external appearance, and twenty rootlets, selected at random from each age-group, were examined microscopically. The black sectors of each circle represent the proportion of infected rootlets.

Differences in the number of entry points recorded in strawberry roots are shown in Table 2.

In this set of plants the density of the extra-matrical mycelium was greatest around the roots growing in treatment (*c*) and least around those growing in treatment (*a*).

The effect of different composts on the number of entry points in roots of clonal strawberries, cultivar Royal Sovereign, was observed in an experiment designed to compare mycorrhizal infection in composts containing either farmyard manure (*M*) (7 parts loam: 3 parts manure), or an equivalent amount of artificial fertilizer (*AF*). Within each manurial treatment unsterilized compost (*o*) was compared with a steam sterilized compost (*s*), with steam sterilized compost re-infected with living mycorrhizal roots (*s* + *lr*), and with dead mycorrhizal roots (*s* + *dr*).

Table 2. *Variation in number of entry points in strawberry roots grown under different conditions and infected by different methods*

Growth environment	(*a*) In the field	(*b*) In autoclaved soil in plugged test tube	(*c*) In autoclaved soil in glasshouse pot
Method of inoculation	Natural soil	*Endogone* spores	*Endogone* sporocarps
Entry points/mm. root length (mean of ten roots)	4·5	5·5	7·6

Sig. diff. ($P = 0{\cdot}05$) 2·3

The following eight treatments were included:

(1) (*Mo*) = loam + farmyard manure, unsterilized
(2) (*Ms*) = loam + farmyard manure, steam sterilized
(3) (*Ms* + *lr*) = loam + farmyard manure, steam sterilized + living mycorrhizal roots
(4) (*Ms* + *dr*) = loam + farmyard manure, steam sterilized + dead mycorrhizal roots
(5) (*AFo*) = loam + artificial fertilizer, unsterilized
(6) (*AFs*) = loam + artificial fertilizer, steam sterilized
(7) (*AFs* + *lr*) = loam + artificial fertilizer, steam sterilized + living mycorrhizal roots
(8) (*AFs* + *dr*) = loam + artificial fertilizer, steam sterilized + dead roots.

A method of steam sterilization as practised in some commercial glasshouses was used; the compost ingredients were maintained at 210° F. for 10 min. There were six replicates in each treatment and plants were kept for 15 months. Entry points were recorded on six rootlets, selected by a sampling method, from each plant. Instead of examining each serial section from nearly 300 roots a sampling method was used, which involved recording only those entry points occurring on sections which touched two lines drawn diagonally across the coverslip. The results of this sampling method corresponded reasonably well with a complete count, as can be seen from Table 3.

In view of the high variability between individual rootlets this sampling method seemed adequate. Although it can be used to make a comparison between treatments, it does not automatically provide an absolute measure of the number of entry points per mm. root length. This can, however, be calculated, knowing that unit length of section corresponded to 4 mm. of root surface (linear measurement of epidermis on both sides of the section), that sections were 30 μ thick, and that the average diameter of roots was approximately 0·4 mm. For the eight treatments recorded in Table 4, the number of entry points per mm. root length varied from 2·6 to 21·1.

Table 3. *Comparison of complete count* (a) *with sampling method* (b)

	Number of entry points per unit length of section in ten roots of one plant									
Root:	1	2	3	4	5	6	7	8	9	10
Method (*a*)	12·0	9·3	1·6	5·8	6·0	12·2	0·3	1·9	0·2	0·4
Method (*b*)	13·6	8·5	1·2	5·6	3·3	11·5	0·1	1·2	0·08	0·6

Table 4. *Variation in number of entry points in strawberry roots, cultivar Royal Sovereign, grown in different composts*

	3 (*Ms*+*lr*)	1 (*Mo*)	2 (*Ms*)	4 (*Ms*+*dr*)	5 (*AFo*)	7* (*AFs*+*lr*)	6 (*AFs*)	8 (*AFs*+*dr*)
Mean no. entry points/ unit length section	6·5	3·9	2·6	1·9	1·7	0·9	0·8	0·8

* Only three plants survived in this treatment.
Sig. diff. all treatments excluding no. 7 ($P = 0·05$) 1·2.
Sig. diff. no. 7 and all other treatments ($P = 0·05$) 1·5.

All the plants in the treatments intended as controls, i.e. steam sterilized and steam sterilized + dead roots, became mycorrhizal without a single exception. The ability of vesicular-arbuscular endophytes to survive steam sterilization as practised in some commercial glasshouses is clearly shown by these experiments. It is further confirmed by other experiments, in which the possibility of chance contamination was virtually excluded by growing seedlings in steam sterilized compost in plugged test tubes. Mycorrhizal infections develop only slowly in steam-sterilized soil, but they become increasingly evident after 6 months.

The above record shows that the number of entry points was greater in composts containing farmyard manure than in those with artificial fertilizer. Several explanations of this observation are possible. The fungus may be capable of a more extensive extra-matrical development in composts containing a high proportion of organic matter. The ability of this fungus, when in mycorrhizal association, to accelerate the breakdown of organic matter has been noticed repeatedly during the washing of root systems grown in autoclaved soils with *Endogone* inoculum. When non-mycorrhizal root systems without *Endogone* inoculum are immersed in water for a short time the soil drops cleanly away from the roots, and only a few large pieces of solid organic matter remain impaled on individual rootlets which grow straight through them (Pl. 27, fig. 5*a*). Subsequent changes of washing water remain quite clear. On the other hand, root systems that have become mycorrhizal as a result of inoculation with *Endogone* spores or sporocarps are difficult to wash out and require much shaking to remove adhering soil particles. Even after repeated washing, finely divided particles of organic matter adhere firmly to the roots (Pl. 27, fig. 5*b*), and the washing water continues to be muddy even after several changes. The circumstantial evidence that the break-down of organic matter in these cases is accomplished by the mycorrhizal fungus is strong, because although the sporocarp inoculum undoubtedly contains other soil organisms the spore inoculum is much purer, and where it failed to establish mycorrhizal infection it also failed to show any effect on the organic matter.

If, then, the view is accepted that this fungus in mycorrhizal association plays a part in breaking down organic matter, it would be reasonable to associate the larger number of entry points in the farmyard manure treatments with a more extensive extra-matrical mycelium. Other, less direct, explanations are however also possible, and the condition of the root itself probably acts as a contributory factor. In both apples and strawberries endotrophic mycorrhizal infections are strictly confined to the primary cortex, which is only short-lived. The distinction between the permanent root system, which never becomes mycorrhizal, and the temporary absorbing roots which do, is particularly marked in the tree fruits. Infection of the actively growing root tip rarely occurs, and it has also been observed that, after a certain stage of development has been reached, mycorrhizal roots very rarely contain new infection points. It seems that there is a certain period, probably determined by the rate of suberization of the epidermis and the onset of secondary thickening, which eventually leads to the disintegration of the primary cortex, during which a developing rootlet may be invaded. Any effect of manurial treatment on rate of root growth may affect the period during which a root is potentially susceptible to invasion by the endophyte. Given a certain density of extra-matrical mycelium, it is evident that the longer this stage lasts the higher the number of entry points is likely to be. In addition, the anatomical appearance of the root epidermis suggested that there was some structural difference associated with the different manurial treatments, possibly caused by a change in soil pH or by some effect on the microbial population of the soil. Lastly, it should be pointed out that elongation of rootlets may be affected by manurial treatments, and is fairly obviously affected by mycorrhizal infection. This would tend to give a higher number of entry points per mm. root length in strongly infected roots, which are characteristically shorter and fatter. An attempt to estimate the intensity of infection in different treatments by recording the proportion of intracellular space occupied by the fungus gave the result shown in Table 5.

Table 5. *Intracellular development (recorded in five categories) in mycorrhizal strawberry roots, cultivar Royal Sovereign, grown in different composts*

	3 (*Ms*+*lr*)	1 (*Mo*)	4 (*Ms*+*dr*)	5 (*AFo*)	8 (*AFs*+*dr*)	6 (*AFs*)	2 (*Ms*)	7 (*AFs*+*lr*)
Mean intracellular development	3·5	2·9	2·2	2·1	2·0	1·8	1·8	1·4

Sig. diff. all treatments excluding no. 7 (P = 0·05) 0·84.
Sig. diff. no. 7 and all other treatments (P = 0·05) 1·02.

Category 1 = < 10% of intracellular space occupied by fungus, category 2 = < 25%, category 3 = < 50%, category 4 = < 75%, category 5 = < 100%. The treatments 3 (*Ms*+*lr*) and 1 (*Mo*) with an outstandingly high number of entry points (Table 4) also showed the greatest degree of intracellular infection (Table 5). The significantly high number of entry points in the compost re-inoculated after sterilization in treatment 3 (*Ms* + *lr*) is also interesting as a possible indication of increased development of mycorrhizal fungi permitted by the reduced competition from other soil organisms.

Two other points should be briefly considered. One concerns the viability of hyphal connexions, the other the possibility that some of the entry points may in fact be points of egress. Many 'entry' points show a more or less well-developed appressorial swelling and thus afford clear anatomical evidence that they are in fact places where the fungus has entered the root; on the other hand, clear anatomical evidence for a point of egress was never seen. It is therefore concluded that if egress occurs at all it must be rare. Evidence has been found (Pl. 27, fig. 6) of some thick-walled, apparently viable, vesicles emerging into the soil as a result of disintegration of the surrounding root tissue. A similar conclusion was reached by Schrader (1958) in an examination of mycorrhizal pea roots. Indications obtained from cotton blue staining suggest that such vesicles are the only structures of the endotrophic mycelium which can survive, all other parts of the fungus becoming moribund as the root decays. The question of the viability of hyphal connexions has been repeatedly raised in the literature, and varying views have been expressed. In the writer's experience, connecting hyphae become visibly empty and moribund only when the rootlet starts to decay.

Discussion

Evidence has been presented that in the material investigated hyphal connexion between mycorrhizal roots and the extra-matrical mycelium was not inconsiderable. Figures varying from 2·6 to 21·1 entry points/mm. root length (= approximately 2·1–16·9 per mm.^{2} root surface) were recorded in strawberries, and from 4·6 to 10·7/mm. root length in apples. In two previous investigations McLuckie & Burges (1932) recorded 2·5–4·1 connexions/mm.^{2} root surface in *Eriostemon crowei*, and Lihnell (1939) found 4–16/mm. root length in *Juniperus communis*. The three records agree as to the general order of frequency. In some grass roots Nicolson (1955, 1959) recorded a frequency of one entry point/mm. root length, but found that entry points were much more frequent where penetration was through root hairs rather than epidermal cells. The present study showed that significant differences in frequency of hyphal connexion occurred with different manurial treatments, with different environmental conditions and at different times of the year. To what extent such differences reflect the activity or amount of the extra-matrical mycelium and how far they may be determined by the state of the roots themselves is a matter for conjecture, and it is not improbable, as in the case of root length, that the two are inter-connected. Although it is difficult to prove this other than by observational evidence, the extent of the extra-matrical mycelium was considerable in the material examined. It would, however, be well to remember that the field evidence only applied to roots from one locality, and that the rest was derived mainly from glasshouse plants grown in steam-sterilized or autoclaved soils that were subsequently inoculated.

One of the chief interests in the vesicular-arbuscular mycorrhiza has always centred around the question as to whether the fungus could contribute anything to the nutrition of the associated plant. In so far as any direct contribution is concerned, as distinct from a possible indirect effect of infection on root metabolism, efficiency, longevity or other characteristic, this is only possible if the fungus is able to utilize some additional source of nutrients in the soil. Evidence is accumulating that any independent existence of the *Endogone* sp. in the soil is restricted to survival rather than growth. Two observations suggesting that it may exhibit greater activity as a component of the soil flora when in mycorrhizal association are the effect of mycorrhizal infection on the break-down of soil organic matter, and morphological evidence of the abundant development of the extra-matrical mycelium, with thin-walled haustoria-like branches, in and around particles of organic matter. Although the picture which emerges of a continuous, ramifying, frequently anastomosing hyphal system stretching from organic particles in the soil into the cortical cells of the host root is clear enough, this is not evidence that conduction along this hyphal system occurs. One piece of evidence that some conduction must occur in the opposite direction, out of the root, is the fact that soil inoculation with an *Endogone* sp. has no effect on the organic matter unless a mycorrhizal infection is established. Methods making use of radioactive materials, similar to those which have been used in the study of ectotrophic mycorrhiza, might yield some direct evidence of translocation.

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EXPLANATION OF PLATES 26 AND 27

PLATE 26

Fig. 1. Mycorrhizal apple rootlets and their surrounding extra-matrical mycelium soaked out of the soil.

Fig. 2. Thin-walled, occasionally septate hyphae arising from the thick-walled, permanent hyphal system of the extra-matrical mycelium. A, Enlargement showing origin of thin-walled branches from angular projections of the permanent hyphae.

Fig. 3. Longitudinal section of a mycorrhizal apple rootlet showing the formation of an appressorium at the point of entry.

PLATE 27

Fig. 4. Mycorrhizal strawberry root penetrated at intervals by branches from an extra-matrical hypha growing parallel to the root surface.

Fig. 5. Washed roots of strawberry plants grown in autoclaved soil and peat (*a*) non-mycorrhizal, (*b*) mycorrhizal. The effect of inoculation with *Endogone* spores on the decomposition of peat particles is clearly shown in (*b*).

Fig. 6. Strongly stained vesicles emerging from a disintegrating apple rootlet.

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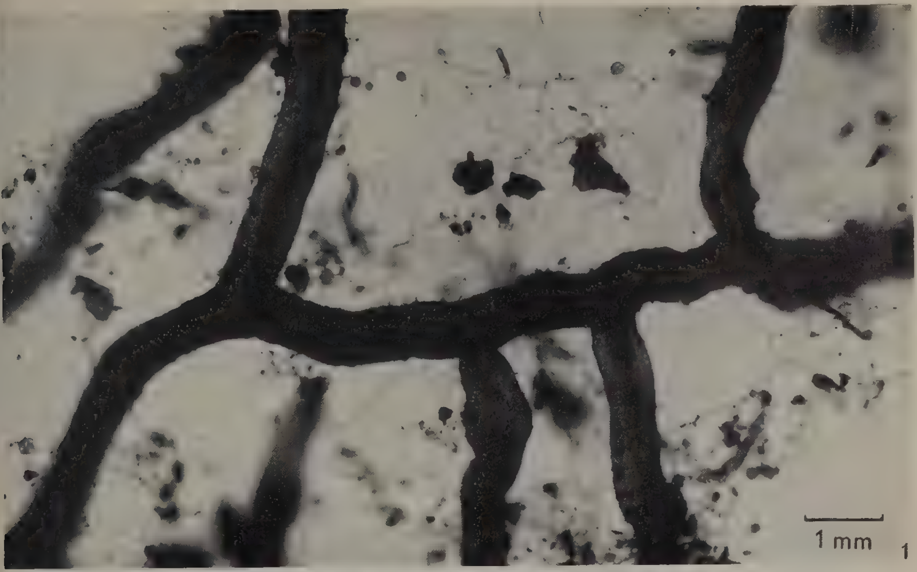

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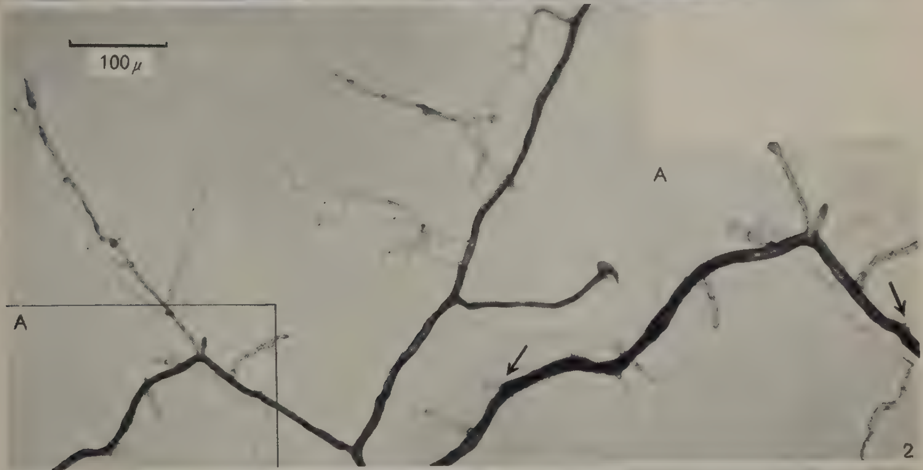

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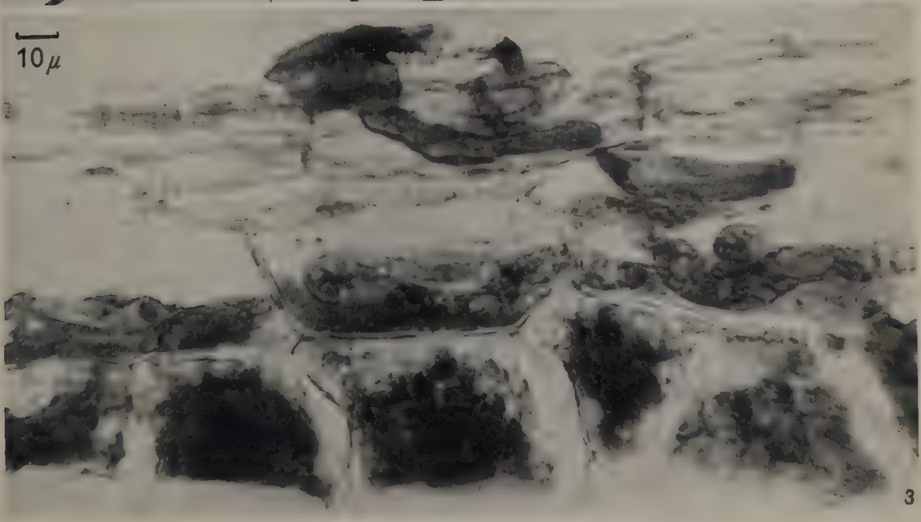

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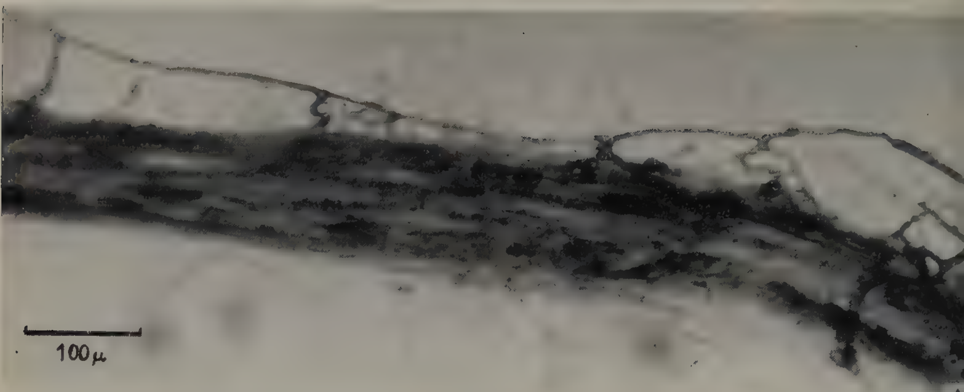

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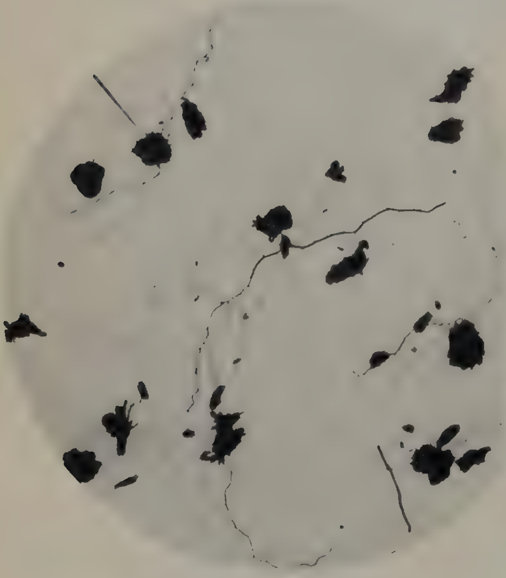

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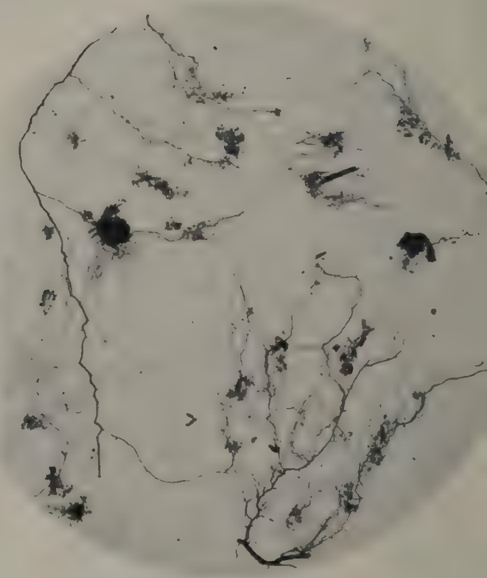

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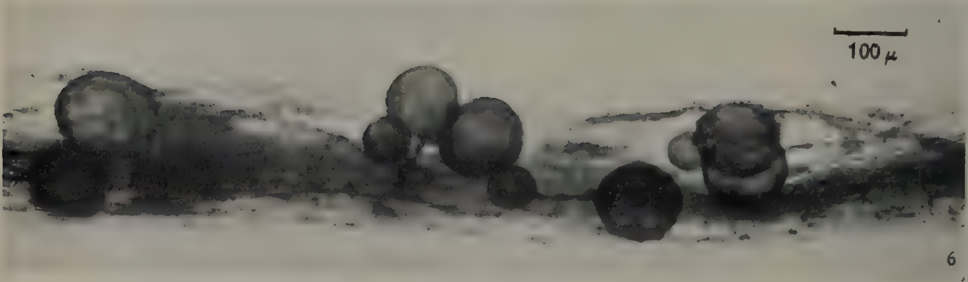

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Trans. Brit. mycol. Soc. **42** (4), 449–457 (1959).

ECOLOGY OF *ENDOGONE*

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(With Plates 28 and 29 and 3 Text-figures)

1. At least four common plants of the swamp flora of Central Alberta contain within their roots a vesicular-arbuscular endophyte of the genus *Endogone*. This fungus (or group of fungi) is not confined to swamps. It occurs in alpine plants and has been found in fossil roots. Its walls are thicker, and its hyphae more complex than those of *Pythium*. 2. From root fragments, *Endogone* grows out into the soil and produces chlamydospores indistinguishable from the storage vesicles of the same fungus found within the root cortex. The chlamydospores are sometimes aggregated into irregular masses which constitute the naked fructifications of *E. fasciculata*. 3. Chlamydospores of *E. fasciculata* are common both in soil and in the stomachs of many species of rodents. The possible importance of rodents (and of insects) in the life history of the fungus is discussed.

INTRODUCTION

In soil in North America there have been found quantities of fungal spores so large as to be almost visible to the naked eye. They are distinctive. Their walls are dark-coloured, sometimes stratified, and as much as 10 μ thick. The cavity of the spore is not cut off from the subtending hypha by a septum, and the wall thickening continues for some distance along the hypha. The spores have been classified as *Endogone*, the most common species being *E. fasciculata* Thaxter.

In Alberta, in the 'blue clay' lying beneath peat bogs and in the mud taken from swamps, clusters of *Endogone* spores were found attached to a thick-walled mycelium which appeared to be growing out of plant roots.

The present paper will describe *E. fasciculata* as it is to be found growing (1) saprophytically in the soil and (2) symbiotically within the roots of swamp plants. It will also discuss the relationship between *Endogone* and rodents.

HISTORICAL

In 1922 Thaxter published a monograph of the genus *Endogone*. Some species produce a definite sporocarp, while others form only an indefinite loosely coherent spore mass. Thaxter's illustration of *E. fasciculata* shows an irregularly lobed naked spore mass made up of thick-walled chlamydospores. He stated that zygospores may or may not be present.

However, a fungus which might be this species was known by its spores much earlier. Butler (1939) cites Walker and West, who, as long ago as 1898, found quantities of large thick-walled spores in soil near the roots of sugar cane.

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In 1955, by a process of wet sieving and decanting, Gerdemann recovered large, spore-like bodies from clover fields in Illinois. Later, Dowding (1955), Dr Jellison (unpublished) and Bakerspigel (1956, 1958), in widely separated regions of North America, found spores resembling those of *E. fasciculata* in soil and in the stomachs of rodents.

Similar spores have also been found as fossils. Rosendahl (1943) isolated them from three Pleistocene deposits in Minnesota, and Butler described them from deposits beneath Alberta muskegs. Kidston & Lang (1921) described almost identical spores associated with the earliest known land plants from Rhynie fossil beds in Scotland.

There is evidence that, aside from its occurrence in soil, *Endogone* enters into a mycorrhizal relationship with plants. Butler has summarized the more important earlier observations made over nearly one hundred years upon mycorrhiza associated with various groups of fungi. Of these, Gallaud's beautifully illustrated thesis (1904) stands out. He described the position in the host cortex and the type of branching of four types of endophytes.

Dangeard (1900) described a non-septate endophyte with terminal swellings, *Rhizophagus populinus* Dang. Balsam poplar roots near Edmonton often contain an endophyte of this type but with thinner walls than those of *Endogone*. Hawker and her co-workers (1957) state that *Pythium ultimum* Trow is a common endophyte of *Allium ursinum* L. and when inoculated on roots of other *Allium* spp. produces mycorrhiza which increase the vigour of the plants. This endophyte may be the one described by Dangeard.

Other phycomycetous endophytes appear to be species of *Endogone*. In mountain bogs in Italy, Peyronel (1937) found minute, pale, gelatinous fruit-bodies of *Endogone* attached by strands to the roots of *Viola palustris* L., while Mosse (1953) traced a similar tiny body, 1 mm. diam., made up of *Endogone* chlamydospores, to a strawberry root. Nicolson (1958) found irregular aggregations of spores resembling those from *Endogone* fruit-bodies connected to the endophytic mycelium of *Festuca*. Mosse (1956) described an *Endogone* which she successfully inoculated into strawberry plants. Her photographs show arbuscules and vesicles of the endophyte in the root and detail of the spore walls.

Butler described a mycelium he believed to be that of *Endogone*, which was hyaline at first, becoming honey yellow; though not actually within plant roots, it was connected to them. It is obviously difficult to observe a mycelium buried within the root cortex. However, the present paper will describe a new technique for the treatment of roots which reveals the endophyte. The method has demonstrated a mycelium in Alberta plants resembling the tropical one described by Butler. The same type of mycelium was found by Jones (1924) in the roots of wheat, maize and peas. A fungus which is so widely distributed merits more detailed study.

MATERIALS AND METHODS

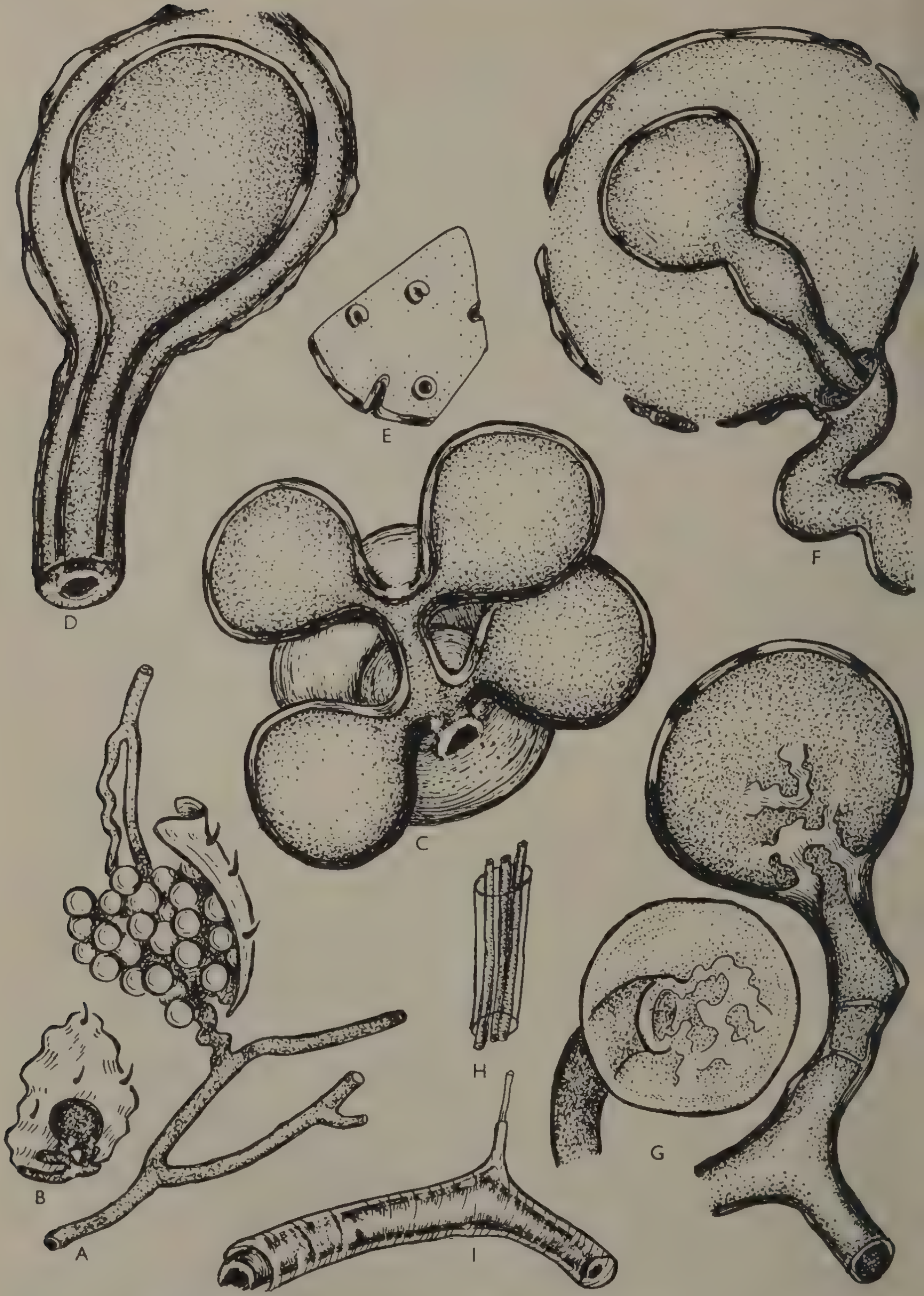
When soil samples and peat profiles from different parts of Alberta were searched for fungus spores, the water and soil mixture was stirred with a needle which was then dragged up the side of the container, carrying with it root-fibres, hyphae and other filaments. In this manner *Endogone* spores were pulled out of the mud attached to long hyphal strands. The roots of plants were collected from mud flats, alpine meadows, river banks, sloughs and peat bogs in Alberta and usually axamined after they were freshly cut from living plants. However, no amount of harsh treatment seemed to destroy the endophyte. It was found just as easily in the roots of dried herbarium specimens and in plants preserved in alcohol.

Roots containing *E. fasciculata* are not usually enveloped in mycelium, nor are they hypertrophied or stunted. The following special treatment is required to reveal their mycorrhizal nature. Root pieces are stored in 10–20 % KOH or in pancreatin. (The latter digests most of the root tissue leaving the endophyte untouched.) After softening the stored roots in 50 % glycerine, each piece is shredded with needles into three or four longitudinal strips. They are cleared, usually with CCl_4, and mounted in lacto-fuchsin (Carmichael, 1955). For permanent preparations the stain is mixed with an equal volume of Gurr's 'Water Mounting Medium'. Sometimes the mycelium is pulled out from the root tissue with needles and transferred without any of the host tissue to the stain.

ENDOGONE IN SOIL

In the mud of Alberta swamps there are spore masses identified as the fruiting structures of *E. fasciculata*. The masses are indeterminate in shape, without periderm, and barely distinguishable from a lump of caked soil. They are to be found between the leaves of mosses and in sheets surrounding roots of sedges (*Scirpus pumilis* Vahl. and *Eleocharis pauciflora* (Lightf.) Link.) which they envelop in a felt-like covering. Fruit-bodies close together show every gradation of compactness and of definition. Some are spherical balls about 0·5 cm. diam. made up of spores embedded in a clear amorphous matrix. Some are irregular lumps made up of spores arranged on short dichotomous branches in grape-like clusters (Text-fig. 1 C shows part of such a ball). Some chlamydospores are borne on loosely branching mycelia and do not merit the term of 'fruit-body' at all (Pl. 28, fig. 2). Groups of spores are sometimes enclosed in buried insect integuments (Text-fig. 1 A). Two or three zygospores have been found associated with the chlamydospores within these membranes (Text-fig. 1 B).

The older parts of the mycelium are brown strands, up to 20 μ wide, coarse enough to be lifted on a needle, resembling fine plant roots (Pl. 28, fig. 3). When such a strand is examined microscopically it is found to be compound. It is composed of a bundle of narrow hyphae inside a wider one (Text-fig. 1 H). The walls of the enclosed secondary hyphae are about 4 μ thick and are pale coloured. The walls of the



Text-fig. 1. *Endogone* from soil. A, a group of chlamydospores within an insect integument; B, a zygote on an insect integument; C, portion of a fruit-body; D, F, intrahyphal regrowth to form secondary internal chlamydospores; E, portion of a chlamydospore wall showing pits due to parasite attack; G, two views of a chlamydospore showing growth of cell wall into central cavity; H, I, two types of compound hyphae. Magnification; A and B, × 50; all others, × 400.

outer primary hyphae are thin and brown-pigmented. The thick-walled hyphae appear to develop by a stretching of the primary cell-walls, as Blackwell (1949) has illustrated in the sporangia of *Phytophthora*.

Branches grow out at right angles and taper to very fine ultimate threads. Septa are not numerous: they are sometimes flat, more often bulged, frequently occurring in pairs.

Chlamydospores vary from 30 μ diam. to about 10 times as large, averaging about 150 μ. Their walls are rough and brown, thin at first, later becoming thick, particularly at the region of attachment of the spore to the hypha. Sometimes the wall-thickening extends into the spore cavity (Text-fig. 1 G). One or more thick-walled chlamydospores are often enclosed in the thinner-walled primary ones (Text-fig. 1 D, F; Pl. 28, fig. 4).

ENDOGONE AND PLANT ROOTS

The following observations demonstrate that *E. fasciculata* grows within roots of *Parnassia palustris* L.:

(1) In soil brought into the laboratory, groups of *Endogone* chlamydospores were found attached to long, thick-walled hyphae, which were traced to root fragments. The hyphae were firmly attached to these roots and appeared to be growing out from them (Text-fig. 2).

(2) Roots after the treatment described above were mounted in lacto-fuchsin. A brilliantly stained endophyte could be seen in the cortical tissue (Pl. 29, figs. 1–3).

(3) By manipulation with needles, a thick-walled mycelium was pulled out from the root cortex. This mycelium bore vesicles and arbuscules. The vesicles were identical with *Endogone* chlamydospores (Text-fig. 3).

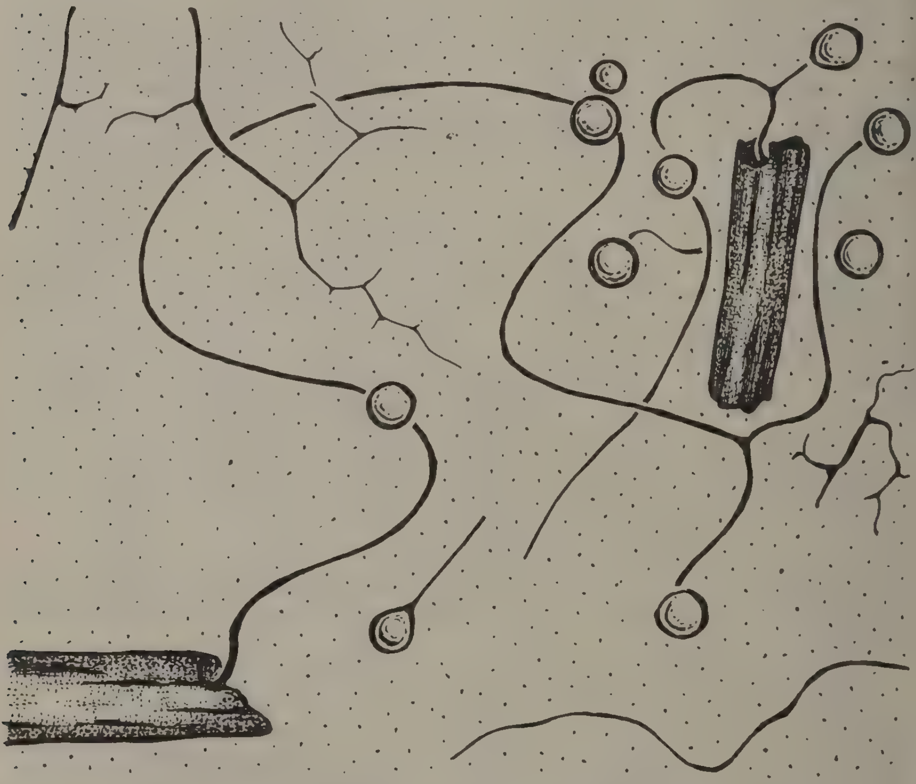
(4) Pieces of root were incubated in water in the manner described by Barrett (1947). A few days later hyphae had grown out from the pieces and had produced terminal vesicles (chlamydospores). After 3 or 4 more days the vesicles had doubled in number.

In Alberta, *E. fasciculata* grows as an endophyte also within the roots of the alpine *Saxifraga cernua* L. and the swamp plants *Scirpus pumilus*, *Eleocharis pauciflora* and *Viola nephrophylla* Greene.

The mycelium in the root resembles that in soil. It produces arbuscules (absorbing organs) and vesicles (storage organs). The latter are identical with chlamydospores produced in soil (Pl. 28, fig. 2); sometimes they are thin-walled with secondary thick-walled vesicles inside (Text-fig. 3, lower right). This figure shows hyphae with attached arbuscules and vesicles; the former are arranged laterally on a hypha which has originated from a compound hyphal strand *ih*.

In the intrusive habit of its secondary growth, *Endogone* shows its relationship to the Devonian *Paleomyces*, an endophyte of the Psilophytales and Asteroxyli. In Rhynie slides, it appears that *Paleomyces* is made up of a thicker-walled mycelium within a thinner-walled one. Its vesicles, which may contain smaller vesicles within, are indistinguishable from the chlamydospores in Alberta soil. For example, Pl. 29, fig. 4, could equally well represent the modern Alberta *Endogone* or its fossil ancestor from Aberdeenshire.

In Alberta glacial deposits *Endogone* is a common root endophyte. The new mycelium grew inside the old one in Pleistocene times as it does to-day.

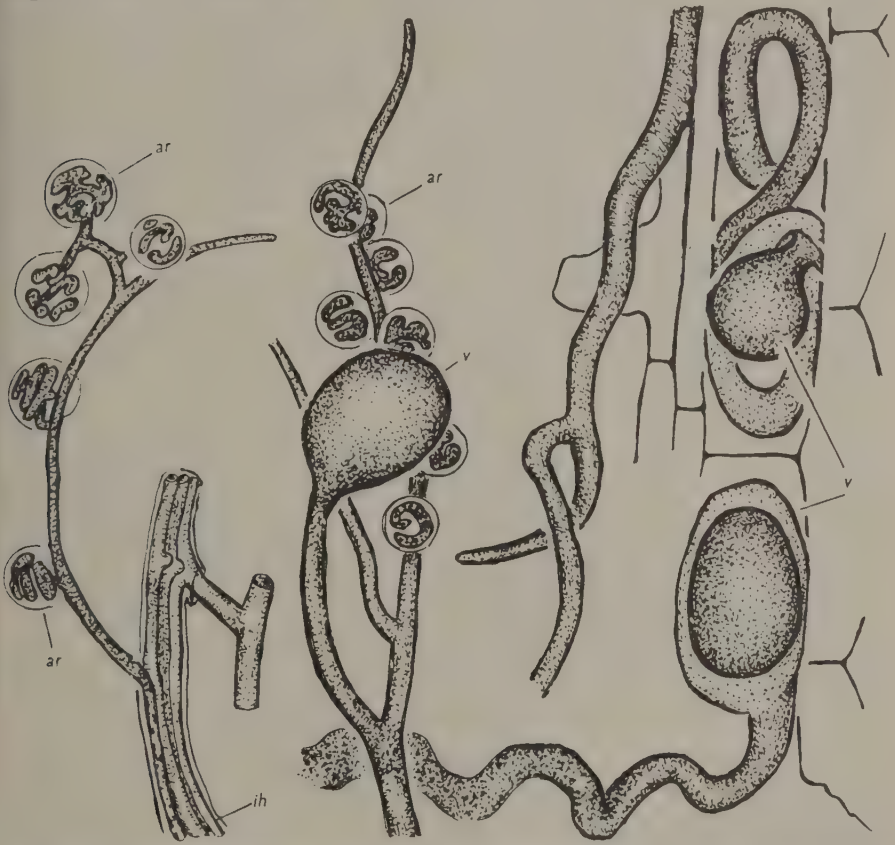


Text-fig. 2. Diagram showing attachment of *Endogone* to roots. (× 50.)

DISCUSSION ON *ENDOGONE* AND RODENTS

Masses of *Endogone* spores have been found in the stomachs of mice, moles, chipmunks, shrews and rock rabbits. These animals were caught in traps set at points ranging from Alaska to Kansas and from Montana to New York and might have been feeding upon underground *Endogone* fruit-bodies before they were trapped. That mice do feed upon underground fruit-bodies, at least on those of the higher fungi, has been shown by Hastings and Moltram in Surrey. They found specimens of the truffle *Elaphomyces granulatus* Fr. with tooth marks of a mouse or squirrel upon them. Photographs of these truffles are reproduced in volume II of Buller's *Researches* (1922).

In 1952, in California, Tevis actually watched chipmunks and ground squirrels dig up and eat the fruit-bodies of gasteromycetes and tuberales. The animals dug up so many that the forest floor was pitted with small excavations. These fungi form mycorrhiza with the roots of trees, and Tevis suggested that rodents, in eating and excreting viable spores, might effect reforestation.



Text-fig. 3. *Endogone* pulled from root tissue. *ar*, arbuscule; *v*, vesicle, *ih*, intrahyphal hypha.

There are apparently no records of rodents seen feeding upon the underground fruit-bodies of the lower fungi. One could easily imagine the nutlike *Endogone pisiformis* Link, which dries to a horny mass shaped like a tiny biscuit, being dug up and stored by small animals. Other species have a pronounced odour that might attract animals.

Most of the spores in animal stomachs in Alberta appear to be *E. fasciculata*, a species with extremely small, loosely coherent fruit-bodies which may never occur abundantly enough in the soil for a rodent to eat. It seems more likely that the animals swallow them unintentionally: for example, they could ingest the spores when feeding upon mycorrhiza. Although Tevis believes that in California roots are of little consequence in the diet of rodents, in Alberta Dr Victor Lewin has observed a muskrat feeding on roots that were emerging through the undercut bank of a canal, and he states that the Alberta pocket gophers (*Thomomys talpoides*) live almost entirely on roots. Another possible source of *Endogone* spores is an insect diet. Thaxter gives some evidence that insects feed upon *Endogone* when he records *Endogone* fragments in the digestive tracts of millipedes. A particularly favoured diet of grasshopper mice in Alberta is carabid beetles. It is possible that rodents eat insects that have been feeding on *Endogone*, or even eat insect remains which have been lying in the soil and into which *Endogone* hyphae have grown and sporulated. Fungus spores associated with the remains of insects are shown in Text-fig. 1 A and B. Bakerspigel (1958) has found *Endogone* spores associated with *Melanogaster* spores in rodents' stomachs. He suggests that the animals have eaten *Melanogaster* fruit-bodies that have been parasitized by *Endogone*.

The Department of Soil Science of the University furnished samples of Alberta soil and of Alberta peat profiles. Mr Tatsuso Yamamoto of Yale University took the photographs here reproduced. The work was made possible by a grant from the National Research Council, Ottawa, Canada.

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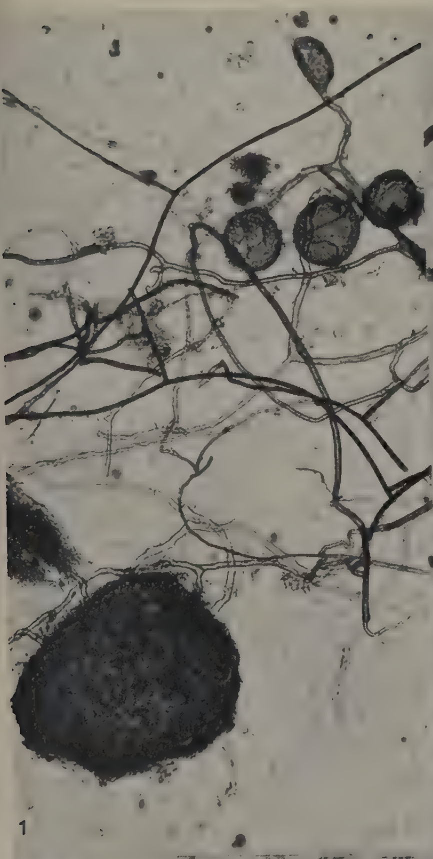

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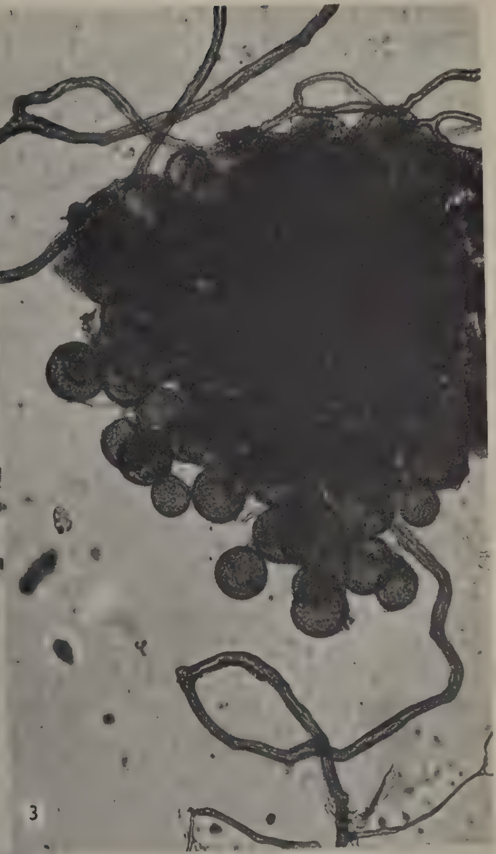

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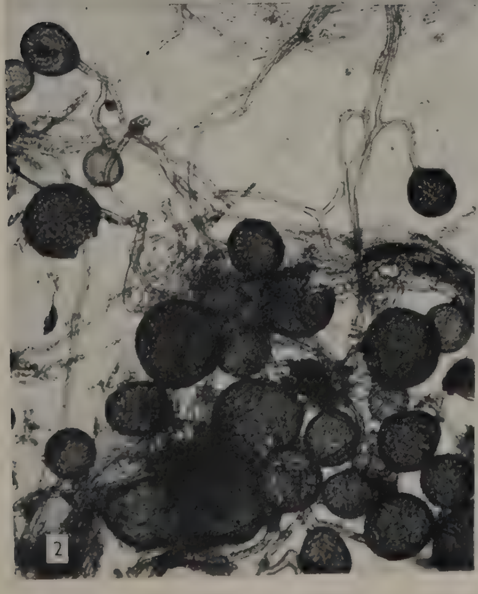

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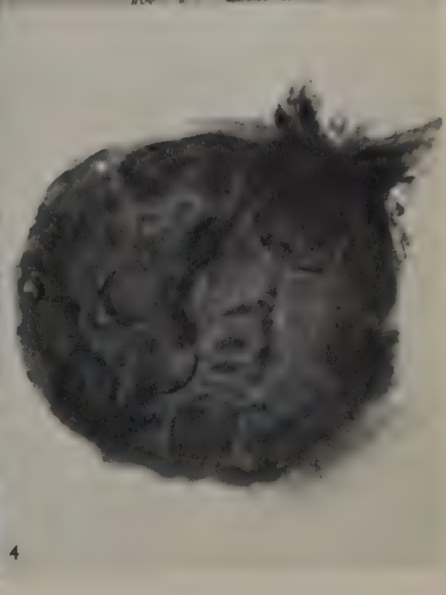

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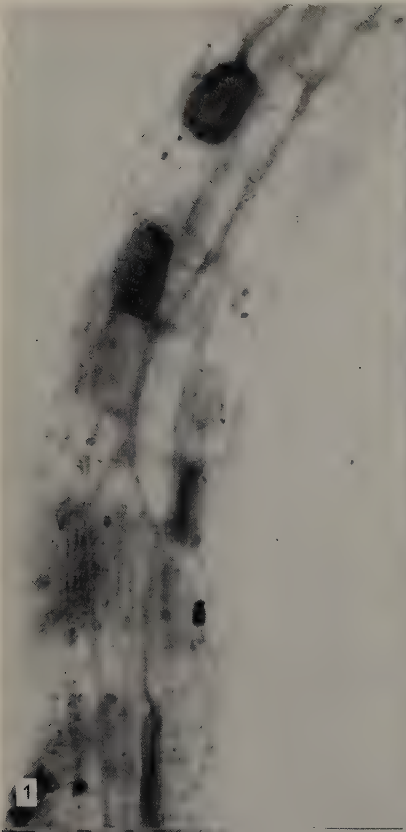

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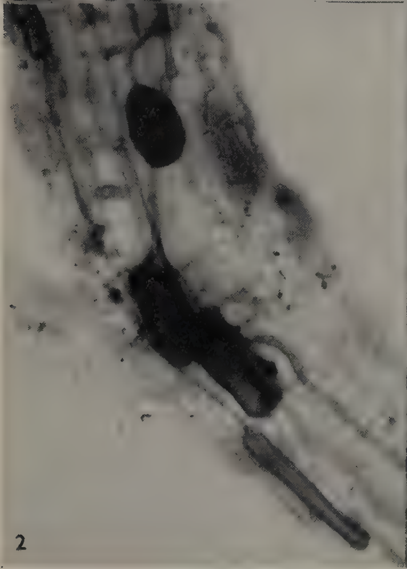

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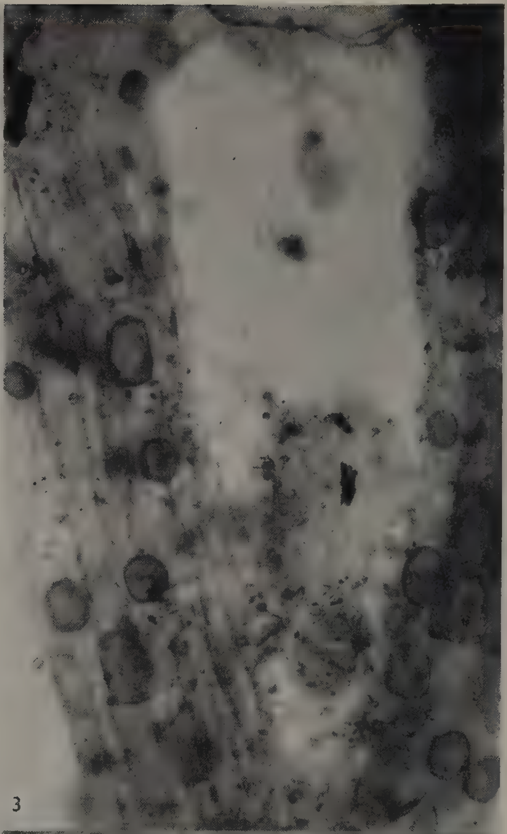

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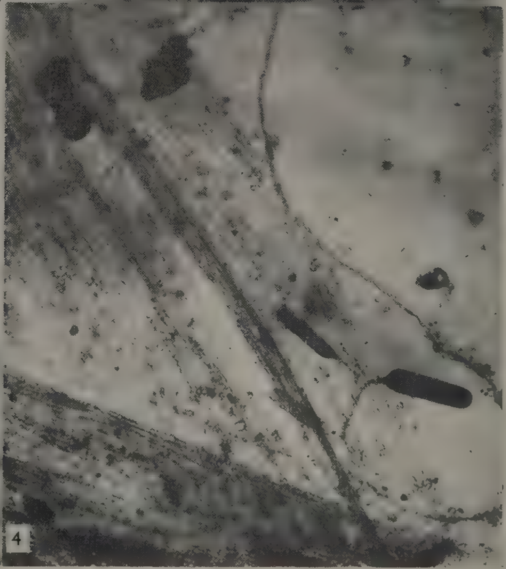

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EXPLANATION OF PLATES 28 AND 29

PLATE 28, *Endogone* in soil

Fig. 1. Slough mud, containing, as well as *Endogone*, one of the higher fungi (darker mycelium). The pear-shaped body (lower-left), firmly attached to an *Endogone* hypha, is interpreted as animal or plant remains; at the lower right, torn ends of *Endogone* hyphae showing their compound nature. (× 100.)

Fig. 2. Mycelium and chlamydospores and the remains of plant roots. (× 100.)

Fig. 3. A stout rhizomorph with a fruit-body or spore cluster. (× 100.)

Fig. 4. Chlamydospore which, through intra-hyphal regrowth, has come to contain at least 8 smaller spores. (× 800.)

PLATE 29, *Endogone* in roots

Figs. 1, 2. *Parnassia* roots.

Fig. 3. Unidentified roots.

Fig. 4. Fossil plants from beneath peat.

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NOTES ON FUNGI FROM NORTH-EAST INDIA

III. *CYLINDROCARPON TENUE* BUGNICOURT (SYN. *GLIOCLADIOPSIS SAGARIENSIS* SAKSENA)

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(With 1 Text-figure)

A comparison of four local isolates of *Cylindrocarpon tenue* with a type culture and also with a type culture of *Gliocladiopsis sagariensis* has revealed little cultural and morphological difference between the two species. *G. sagariensis* is reduced to synonymy with *C. tenue*.

In the course of investigations on the density of the rhizosphere microflora of tea bushes as affected by root rots, very often a hyalodidymous tuberculariaceous fungus was isolated. It was identified as *Cylindrocarpon tenue* Bugnicourt. Many isolates of this fungus were carefully and exhaustively studied in this laboratory for over 2 years. The present communication embodies cultural and morphological studies of only four representative local isolates compared with the type of the fungus obtained from Centraalbureau voor Schimmelcultures, Baarn, Netherlands. Also included in the investigations was the type of another recently described monotypic genus of soil fungus, namely *Gliocladiopsis sagariensis* Saksena, which in the light of our present researches is found to be synonymous with *C. tenue*.

The following are the details of isolates studied in the present investigation.

I. From the rhizosphere of a tea bush infected by *Fomes noxius* Corner, Nagadhulie Tea Estate.

II. From tea root infected by *Ustulina zonata* (Lév.) Sacc., Tocklai campus.

III. From decaying tea flowers, tea seed garden, Tocklai campus.

IV. From soil, Tocklai campus.

C. tenue, subculture of Bugnicourt's original isolate from Centraalbureau voor Schimmelcultures.

G. sagariensis, subculture of Saksena's original isolate from Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi.

Isolates were cultured on ten different media, namely, potato dextrose, potato sucrose, Czapek-Dox, Czapek-Dox with yeast extract, carrot, oat, malt extract and soil-extract agars, cooked rice and sterilized tea stem bits. Of all the media used, cooked rice was particularly suitable as all the isolates behaved similarly; therefore a general account of the morphology of different isolates on that medium is given. Cultural characters were recorded after 15 days' growth, measurements of spores, conidiophores, phialides and chlamydospores being recorded in one-month-old cultures.

For the sake of brevity, the range of cultural and morphological variation of all the four local isolates on cooked rice is presented together under the caption 'Tocklai Isolates'. All measurements are based on a minimum of 100 readings (Table 1).

Table 1. *Dimensions in μ of the different isolates on cooked rice*

	Conidia	Conidiophores
Tocklai Isolate I	12–16(–18) × 2	110–180(–220) × 3–6(–8)
Tocklai Isolate II	10–14(–16) × 2	40–200(–250) × 3–5(–6)
Tocklai Isolate III	10–18(–20) × 2	100–200(–300) × 3–5(–8)
Tocklai Isolate IV	14–18(–22) × 2	40–180(–240) × 3–6(–10)
G. sagariensis	10–18(–22) × 1–2(–3)	140–250(–300) × 4–8(–10)
C. tenue	10–20(–24) × 1–2(–3)	60–140(–200) × 3–6(–8)
	Phialides	Chlamydospores
Tocklai Isolate I	12–14(–16) × 2–3(–4)	14–16(–18)
Tocklai Isolate II	10–14(–16) × 3–4(–5)	16–18(–20)
Tocklai Isolate III	10–16(–18) × 2–4(–5)	16–20(–22)
Tocklai Isolate IV	10–14(–18) × 2–4(–5)	10–16(–18)
G. sagariensis	12–14(–16) × 2–3(–4)	10–14(–18)
C. tenue	10–14(–16) × 2–3(–4)	10–14(–18)

CULTURAL AND MORPHOLOGICAL CHARACTERS ON COOKED RICE

Tocklai isolates. Growth abundant, aerial mycelium effuse, ropy, white to twine (14B3)* or Dogwood (14E8); sporulation sparse to heavy, colour produced on the substratum burnt umber^{P} (15A12), olive green (15L4), Biskra (16A12) or beetle (16J6); chlamydospores and sclerotia present on all isolates.

G. sagariensis. Aerial mycelium abundant, effuse, coremiform, white, turning old ivory (12C3) with the production of chlamydospores; sporulation heavy, pionnotal; colour on the substratum, burnt umber^{P} (15A12) to Biskra (16A12); chlamydospores and sclerotia abundant.

C. tenue. Aerial mycelium not so abundant as in *G. sagariensis* or Tocklai Isolates; white, lanose, forming abundant sclerotia with the ageing of the culture; colour on the substratum Log Cabin plus (15A5); chlamydospores and sclerotia abundant.

Cultural characters of all six isolates were not much different on the various media, as far as the colour of the aerial and substrate mycelium was concerned. On most of the media growth was fairly abundant, floccose, often coremiform with the exception of the type culture of *C. tenue*, which generally produced sparse aerial mycelium on all media except Czapek-Dox agar plus yeast extract. Soil-extract agar was found to be not favourable for mycelial growth nor for sporulation of the culture. The type culture of *C. tenue* was the most prolific in producing spores, followed by Tocklai Isolates. Conidial production was noticeable even on the third day in all isolates irrespective of the media. With ageing of the culture zonation became apparent. Conidia, in all instances, were produced at the ends of conidiophore penicilli in slimy balls which became aggregated to form gelatinous effuse sporodochia in which were imbedded the conidia and conidiophores. It was obvious that conidial production is typically pionnotal as in species of *Fusarium*. Conidiophores were observed to arise from the repent substrate mycelium or as laterals from the aerial hyphae. They were generally branched in an irregular dichotomous or trichotomous fashion, the ultimate branches bearing usually three phialides (Fig. 1 C, G, K). The type of *C. tenue* was observed to produce in some instances a bunch of five to six phialides on oat agar (Fig. 1 L). Conidia are typically cylindrical with an equatorial septum and obtuse ends. Slightly curved conidia were seen in all isolates, being more

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* These figures refer to colour designations in Maerz and Paul's *A Dictionary of Color*, McGraw-Hill, New York (1930).

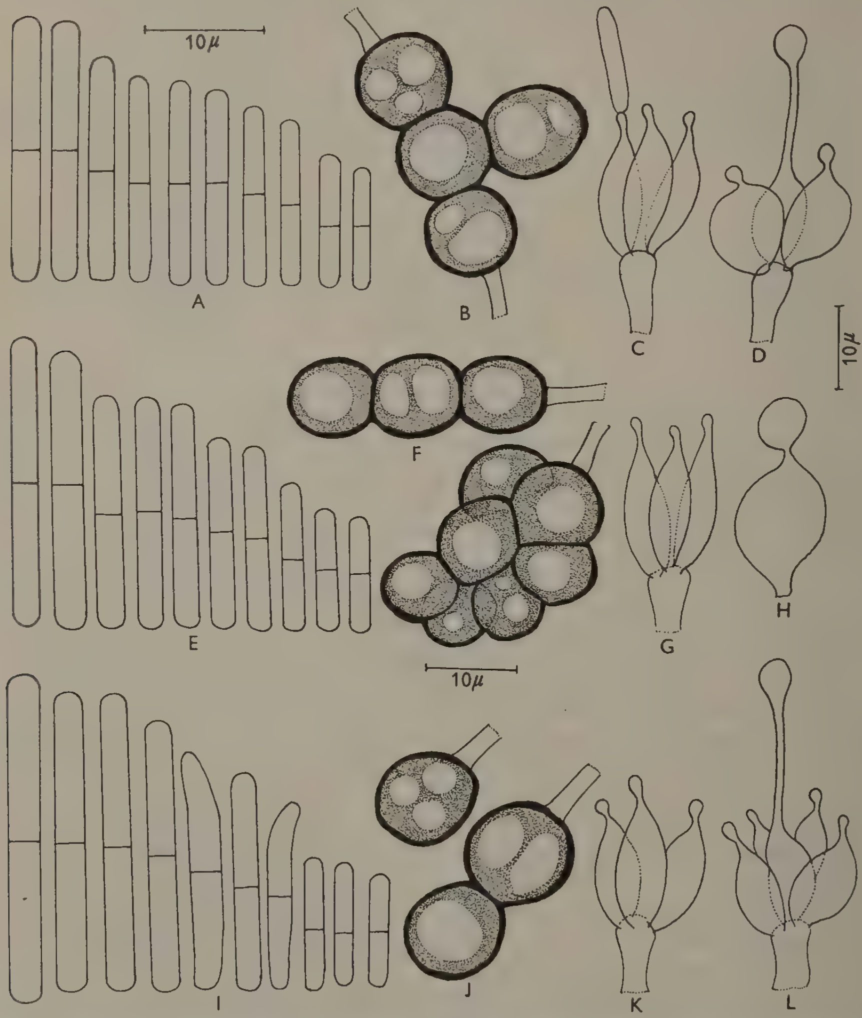

Fig. 1. A–D, type of *Gliocladiopsis sagariensis* Saksena; E–H, Tocklai isolates of *Cylindrocarpon tenue* Bugnicourt; I–L, type of *C. tenue*. A, E, I, typical conidia are illustrated showing the range of size and form; B, F, J, chlamydospores; C, G, K, normal phialides; D, H, L, abnormal phialides on malt-extract agar. All figures, except D, H and L, are made from cultures grown on cooked rice.

common in the type of *C. tenue* (Fig. 1 I). Production of chlamydospores and sclerotia by different isolates was rather desultory on different media, all cultures producing them invariably on cooked rice. The range of variation in conidial dimensions observed on different media is 10–22(–24) × 1–2(–3) μ. Conidiophore length was highly variable in different isolates. Phialides were normally found to measure 10–16(–20) × 2–6(–10) μ. Abnormal phialides were common in all isolates on 2-month-old cultures of oat and malt-extract agars (Fig. 1 D, H, L).

Discussion

The monotypic genus *Gliocladiopsis* was described by Saksena (1954) as a new member of the Moniliaceae with *G. sagariensis* as the type species. The type was isolated from soil samples from Ghatera forests in the Madhya Pradesh, India. The author, when proposing the genus, compared it with *Penicillium*, *Gliocladium*, *Cylindrocladium* and *Candelospora*, but surprisingly made no mention of *Cylindrocarpon.* Contrary to the statement of Saksena, in the present investigation 1-month-old cultures of *G. sagariensis* produced chlamydospores abundantly as well as sclerotia on cooked rice, but only sparsely on Czapek-Dox with yeast extract and potato-dextrose agars. In all the media used the fungus formed irregularly trichotomous conidiophores with well-differentiated banana-shaped phialides. The conidia are formed at the ends of phialides singly and successively, not infrequently in chains, and remain aggregated in slimy balls. Conidial aggregation is a feature common to many hyphomycetes and in the experience of the writer is also found in *Cylindrocladium.* Boedijn & Reitsma (1950) have indeed drawn attention to this feature while re-describing the generic characters of *Cylindrocladium* thus: 'Conidia solitary on phialides, cylindrical, one to many septate, mostly glued together by a colourless substance'. All the above-mentioned features are common to *Cylindrocarpon tenue* also.

Cylindrocarpon was erected by Wollenweber (1913) as a phragmosporous genus of the Tuberculariaceae in the Fungi Imperfecti. It differs from *Fusarium* in forming cylindrical or allantoid conidia which are not pedicellate. It is very near *Cylindrocladium* but the latter has always been described as having a sterile spear head included in the conidiophore penicillus. *Cylindorcarpon* has been classified into two sections, namely, Ditissima with species which are associated with aerial parts of plants and section Chlamydospora which on the contrary have representatives that are generally subterranean. *C. tenue*, according to its author, belongs to the latter section. Bugnicourt (1938) illustrated and gave an exhaustive description of the cultural characters of this fungus and compared it with *C. gracile* Bugnicourt. He clearly states under *C. tenue* in a footnote that his fungus has conidiophores which are di- as well as trichotomous. Besides, the author, while describing the micropionnotes of *C. tenue*, mentions in the footnote on page 175, 'Micropionnotes: voir *Cylindrocarpon gracile* Bugn., note infrapaginale'. His description of micropionnotes of *C. gracile* on page 162 runs: 'Les conidies se formant sur des rameaux fortement dichotomes et dressés, nous désignons par micropionnotes les minuscules globules sphéroïdes de conidies agglutinées constitués sur la partie sommitale de bouquets de conidiophores (facies *Gliocladium*)'. The description leaves no doubt as to the identity of *C. tenue* with *G. sagariensis*, in the nature of production of conidial aggregates. Besides, the dimensions of conidia, conidiophores and phialides of all the local isolates on different media are similar to those of the type of *G. sagariensis*. In view of the striking similarity between these fungi, both culturally and morphologically, it is felt that the name *Gliocladiopsis* is unwarranted and is reduced to synonymy with *Cylindrocarpon*.

It may be of interest to mention here that all the isolates used in the present investigation closely agree in cultural characters, conidial and conidiophore measurements, with those given by Boedijn & Reitsma (1950) for *Cylindrocladium parvum* Anderson. An isolate of this fungus obtained from Centraalbureau voor Schimmelcultures sporulates rather tardily on all media. In the writer's experience many isolates of *Cylindrocladium* obtained from soil produced sterile spear-heads only in the first few subcultures. It may be noted in this connexion that many isolates of *Cyclindrocarpon tenue*, both the type culture as well as local strains, produced short spear-head-like, elongated, sterile phialides in the conidiophore penicillus, particularly on malt-extract agar (Fig. 1D, L). Further work on cultural conditions inducing the production of sterile spear-heads in *Cylindrocarpon* may be necessary before any conclusive affinity can be established with *Cylindrocladium*.

I am grateful to the Director, Tocklai Experimental Station, for permission to publish this paper and to Mr K. C. Sarmah, Mycologist, for his encouragement. I wish to record my indebtedness to Mr E. W. Mason, Drs M. B. Ellis and G. C. Ainsworth of the Commonwealth Mycological Institute and Miss Agathe L. van Beverwijk for their valuable suggestions in the preparation of this paper, to Prof. Dr Johanna Westerdijk of Centraalbureau voor Schimmelcultures, Baarn, and Dr R. S. Vasudeva of the Indian Agricultural Research Institute, New Delhi, for supplying the type cultures used in the study. Technical assistance of Messrs H. K. Phukan, G. C. S. and K. C. Barua is much appreciated.

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ON *ASCOCHYTA ACORI* OUD. AND *A. TYPHOIDEARUM* (DESM.) COMB.NOV.*

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(With 6 Text-figures)

Ascochyta acori Oud. found on dead leaves of *Acorus calamus* L. and *Ascochyta typhoidearum* (Desm.) comb.nov. on dead leaves of *Sparganium ramosum* Huds. are described and illustrated. The nomenclatural position of the fungi is discussed. Behaviour of single spore isolates on agar media and on sterilized 'host' leaves is briefly noted.

INTRODUCTION

Both *Acorus calamus* and *Sparganium ramosum* are rhizomatous, herbaceous perennials which grow in shallow water at the edges of ponds and slow-flowing rivers. *A. calamus* is one of the first waterside plants to die down in the autumn, and by the middle of October in southern England the large outer leaves have usually turned brownish white. They are soft and decay rapidly, especially when submerged, and by December most of the previous season's growth has disappeared. The fungus on *A. calamus* described here appears to be saprophytic, since pycnidia can be found only during the short period in the autumn after the leaves have died and before they have disintegrated. In contrast, the long leaves of *S. ramosum* die in the autumn but often remain intact, though submerged, right through to the following spring. The pycnidia of the fungus on *S. ramosum* can be found in quantity over the whole of this period.

Although the dead parts of many other marsh plants have been examined, these two fungi have been found only on their respective 'hosts'.

ASCOCHYTA ACORI OUD.

A. acori has been found a number of times between October and December when abundant dead leaves of *Acorus calamus* have been available. The pycnidia occur mostly on both exposed sides of the lower parts of the leaves and sometimes on the adaxial surface of the basal sheathing portions when these have been exposed. They may be gregarious or more or less scattered and though sometimes confined to rather definite areas these are not recognizable as distinctly coloured patches (Fig. 1 A).

The pycnidia are completely immersed and, though spherical when young, mostly ellipsoidal with the long axes parallel to the leaf veins (Fig. 1 B). This is apparently due to the limitations imposed on uniform expansion of the developing pycnidium by the network of cells which

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* Part of a thesis approved for the degree of Ph.D. in the University of London.

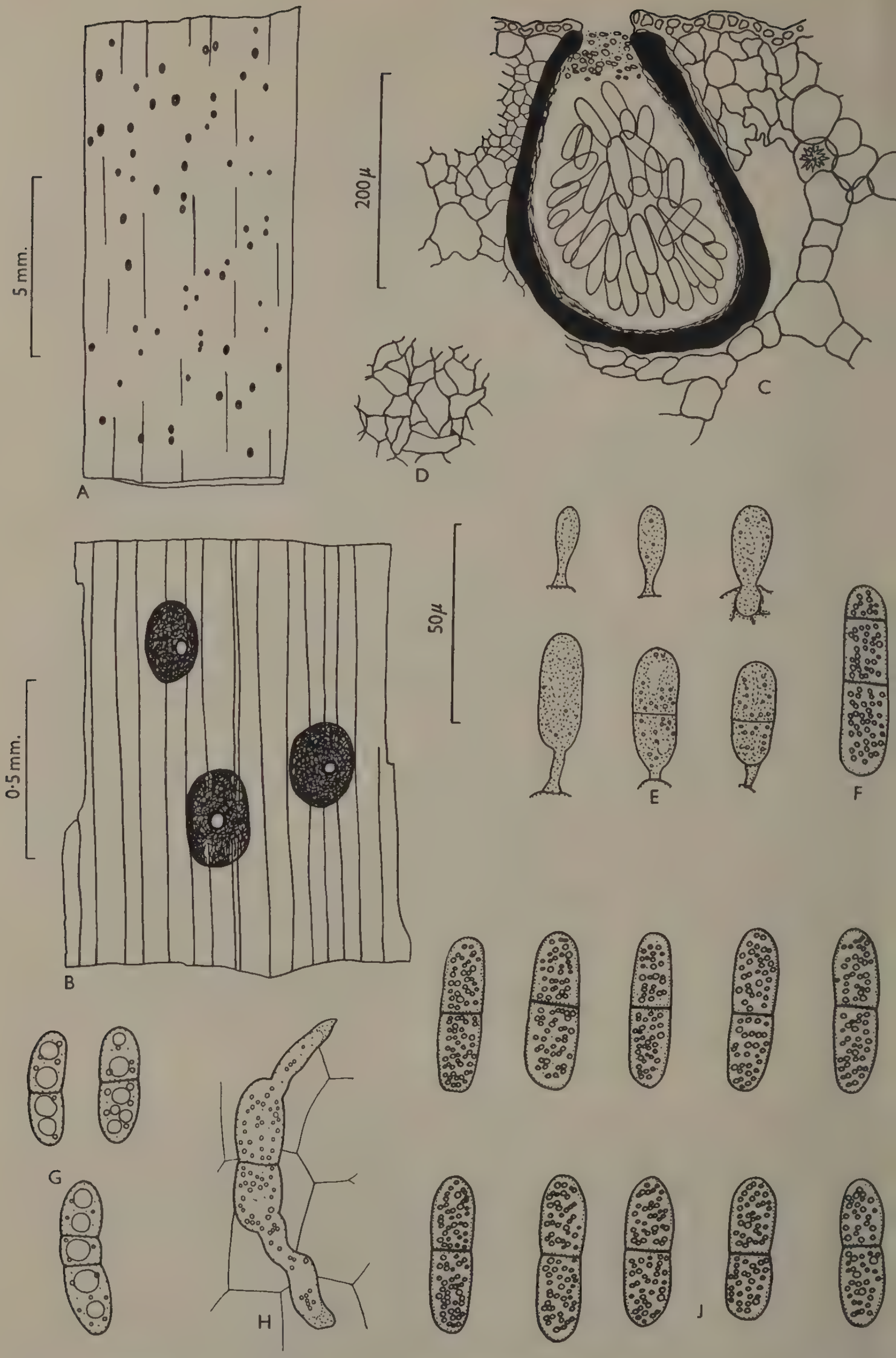

Fig. 1. *Ascochyta acori.* A, part of leaf of *Acorus calamus* with pycnidia (×5); B, part of same (×50); C, vertical section of pycnidium (×150); D, part of outer wall of pycnidium; E, young attached spores in various stages of development stained in 0·01 % aqueous methylene blue; F, atypical 2-septate spore from nature; G, three living spores, the lower 2-septate, from an old pycnidium from nature; H, spore germinating on a submerged piece of *A. calamus* leaf; J, ten random living spores from nature. (D–J, ×555.)

divide the central part of the leaf into narrow longitudinal air spaces. The pycnidia measure 130–340 × 90–240 (average of 50, 206 × 136) μ and have obvious circular ostioles 20–40 μ diam. The wall is 14–25 μ thick (Fig. 1 C) and composed of a few layers of flattened cells. The outer cells are irregular in shape and size, 2–15 μ across, and have irregularly thickened, light-coloured anticlinal walls (Fig. 1 D). They give the crushed pycnidial wall a Honey Yellow,* Yellow Ochre or Buckthorn Brown colour when viewed by transmitted light under low power of the microscope.

The young spores are formed as projections of the inner cells of the wall (Fig. 1 E). They may be borne on short stalks and often become septate before they are detached.

Within the pycnidium the spores are embedded in a thick mucilage containing a number of small oil drops. The spores (Fig. 1 J) are colourless, oblong-cylindrical, 3–4 times as long as broad, usually straight and have rounded ends. There is a single obvious septum which may be median but is often nearer one end of the spore, dividing it into two unequal cells. Only twice have abnormal 3-celled spores been seen (Fig. 1 F, G). The smooth lateral walls are more or less parallel and though they are not constricted at the septum when the spores are freshly mounted they soon appear so due to the intake of water and consequent bulging of each cell. Healthy spores contain many small, evenly sized oil drops; in old ones these may have coalesced to 2–4 large guttules (Fig. 1 G). This is contrary to the statement of Diedicke (1915) suggesting that each spore has four oil drops at first which later break up.

Dimensions of 100 spores taken at random from two different collections

Length (μ)			Width (μ)	
Range	Mean	Standard deviation	Range	Mean
31–47	38·4	2·9	9–12	10·3
26–40	33·7	3·06	8·5–13	11·0

Oudemans (1898) described *Ascochyta acori* on *Acorus calamus* from Holland with pycnidia about 170 μ broad and spores oblong-cylindrical, straight, rounded at both ends, scarcely constricted at the septum, 4-guttulate. No spore measurements were given and neither Allescher (1901) nor Saccardo (1902) provided any additional information. However, Diedicke (1915), describing material of this species from Germany, stated that the pycnidia measured up to 300 μ diam. and that the spores were oblong-cylindrical, mostly straight, rounded at both ends, 1-septate, not constricted, with 4 oil drops, later granular, and 30–40 × 10–12 μ. Migula (1921) quoted the same information but also published a figure of the spores. The pycnidial and spore dimensions of my material are very similar to these and there can be little doubt that this fungus is *Ascochyta acori* Oud. even though I have not been able to see any type material.

Ellis (1913) gives the first alleged British record of this species and describes the pycnidia as minute, scarcely recognizable except with a lens and the spores as more or less cylindrical, rounded at each end, 2-celled, slightly granular, 25–28 × 7–10 μ. I have examined this specimen in the Herbarium of the Royal Botanic Gardens, Kew (Mollington Canal, Wirral, Cheshire, 18/5/1912). It consists of distal parts of leaves of *Acorus calamus* which were obviously living when collected. I can find no pycnidia on the few small brown patches on these but in view of the minute size they are stated to be, and the spore sizes given, it is very doubtful whether Ellis's fungus is the same as the one dealt with here.

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* All colour designations are according to Ridgway (1912).

A certain amount of confusion has arisen because two years earlier than Oudemans's publication Bresadola (1896) described *Stagonospora calami* on *Acorus calamus* from Germany. The pycnidia are given as 200 μ diam. and the spores 24–30 × 8–9 μ, 1- to 3-septate, and 2- to 4-guttulate. So far I have been unable to see type material, but the spore dimensions are below the ranges I have found in my material. However, Grove (1935) uses Bresadola's name and reduces Oudemans's species to synonomy with it. He says in a footnote that Oudemans's and Diedicke's...'descriptions read as if they referred to a young *Stagonospora* allied to *S. typhoidearum*, which no doubt the "*Ascochyta*" is'. My material fits the descriptions given by Oudemans and Diedicke and not that of Bresadola. In my experience, but for very rare exceptions (Fig. 1 F, G) the spores are 1-septate and therefore belong to an *Ascochyta* and not to a *Stagonospora*. Further supporting evidence for this contention is provided by the cultural features of my isolates of this fungus, which are very different (e.g. in slow growth rate and irregular colony formed) from those of any *Stagonospora* I have grown in pure culture.

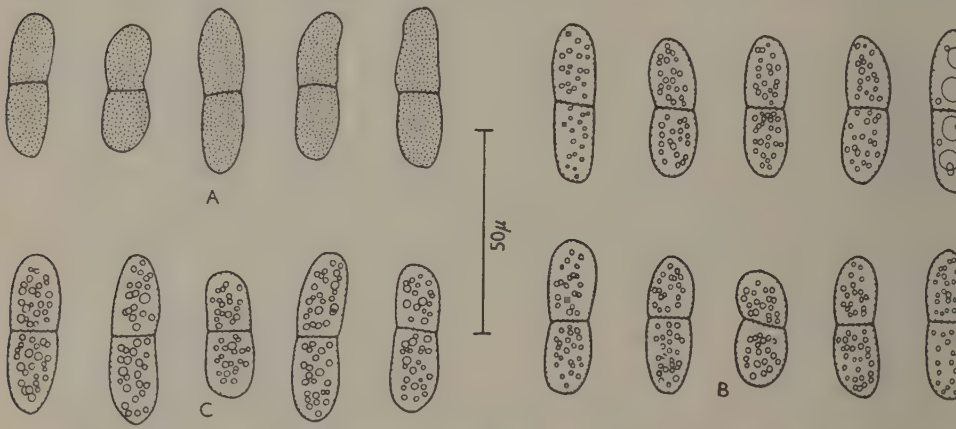

Fig. 2. *Ascochyta acori*. A, five random spores (on leaves of *Acorus calamus*, Great Heywood, Staffs, August 1929, coll. W. B. Grove) mounted in lactophenol with cotton-blue; B, ten random living spores from pycnidia produced on re-infected sterilized *Acorus* leaves; C, five random living spores from pycnidia produced on re-infected sterilized *Carex riparia* leaves. (All × 555.)

In Kew Herbarium a collection labelled *S. calami* (on *Acorus calamus*, canal, Great Heywood, 9/5/1929) and bearing a note 'Apparently new to Britain, conf. Grove', is, in fact, on *Sparganium*. I have examined part of this same collection from Grove's herbarium and there is no doubt that the host is *Sparganium* and the fungus the one dealt with in the next section. There is another specimen of Grove's on the same sheet and from the same locality (Great Heywood, Staffs, August 1929) bearing the label *A. acori* Oud. = *S. calami* Bres. The host is correctly identified and the fungus on it is obviously the same as mine. The pycnidia are characteristically ellipsoidal and contain the typical spores of this species (Fig. 2A). The dimensions of 50 random spores are: length 30–39 (mean 34·9) μ; width 9–11 (mean 10·1) μ. This is therefore the first correct British record of *Ascochyta acori*.

The spores germinate fairly quickly in water at room temperature, a single germ tube being formed from near the distal end of each cell (Fig. 1H). On 2% malt-agar growth is poor, and a very slow growing, irregular-shaped colony is formed which causes the medium to turn Wax Yellow to 5 mm. beyond its edge. A Pearl Grey, felted aerial mycelium is formed, mostly on the inoculum. The reverse is Dark Mouse Grey masked by the stained medium. Growth is more extensive on potato dextrose and oatmeal agars, the colonies being irregular in shape with uneven, ragged edges. The aerial mycelium is uneven, loosely felty, Light Olive Grey to Deep Olive Grey. The reverse is Fuscous Black to black. Old colonies on 2% malt agar have a uniform Pale Olive Grey, felted aerial mycelium and are Fuscous Black in reverse, whereas on potato dextrose and oatmeal agars the aerial mycelium is felty, fairly uniform and varies in colour from Pale Olive Grey to Dark Olive Grey. The reverse is Fuscous Black. Pycnidia have not been observed in cultures on the three media used.

On sterile pieces of *Acorus calamus* leaf a thin surface mycelium is formed. After about 2 weeks abundant typical pycnidia appear both above and below the water surface. They contain many typical spores (Fig. 2B). Similar growth and fruiting also takes place when the fungus is grown on sterilized pieces of *Carex riparia* leaves (Fig. 2C).

The dimensions of 100 spores taken at random from two different cultures are:

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Re-infected *Acorus calamus*	26–40	32·6	2·97	9–12	10·2
Re-infected *Carex riparia*	28–45	37·5	2·84	10–12·5	10·9

Specimens have been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 75295), and the Herbarium, Royal Botanic Gardens, Kew.

ASCOCHYTA TYPHOIDEARUM (DESM.) COMB.NOV.

This species has been found many times on the dead leaves and also on the stems of *Sparganium ramosum*. The pycnidia occur mostly on the outer surfaces of the trigonous leaves but also appear on the adaxial surfaces where these are exposed. They are more or less gregarious although often distributed uniformly along a considerable length of the leaf (Fig. 3A, B). Contrary to the statement made by Grove (1935) the pycnidia are not confined to distinct yellow spots, though the fungus is usually most prevalent when the uniform colour of the dead leaves is Soyal Brown to Snuff Brown.

The pycnidia are black and conspicuous though completely immersed in the tissue with the apical region often lying in a slight depression of the leaf surface (Fig. 3E). They range from 110 to 255 μ diam. (average of 50, 191 μ) with ostioles 15–27 μ across. Although the larger ones are spherical (Fig. 3E) many smaller, irregularly-shaped ones are found, especially near the abaxial ridge of the trigonous leaf (Fig. 3C, D). In this region the leaf is more compact with few air spaces and therefore uniform expansion

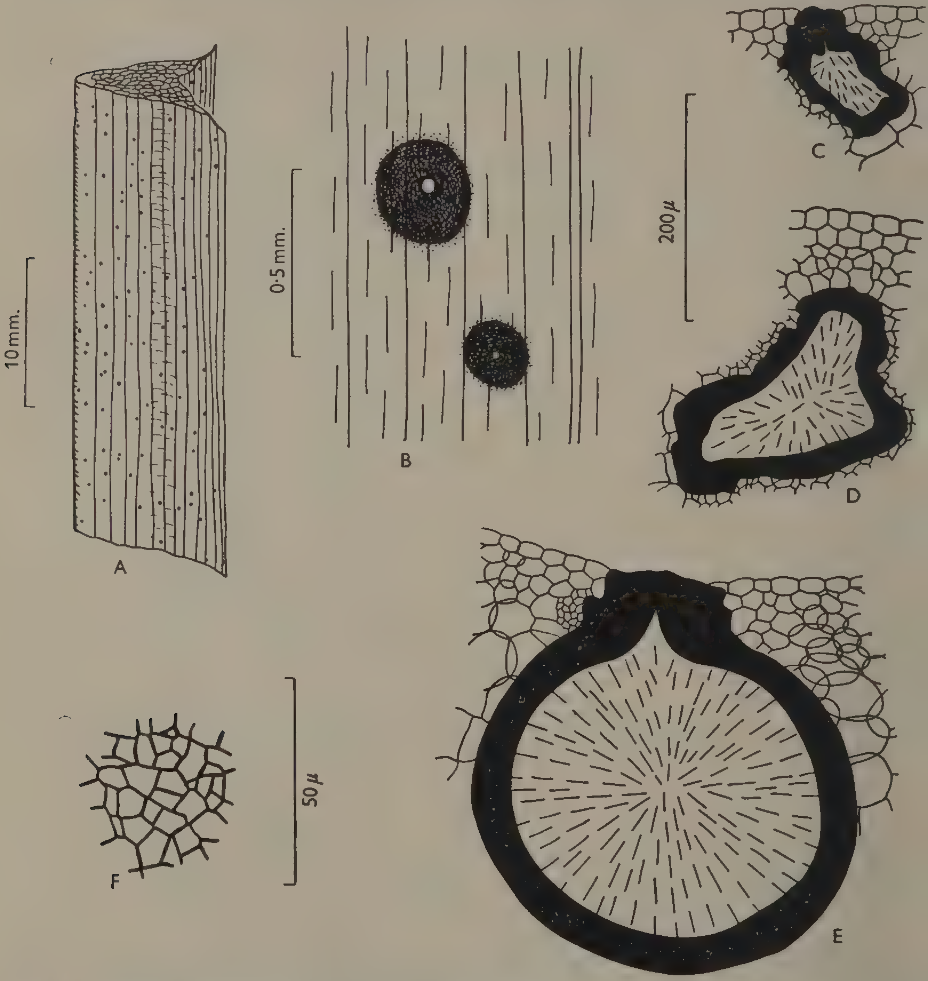

Fig. 3. *Ascochyta typhoidearum.* A, part of leaf of *Sparganium ramosum* with pycnidia (× 2); B, part of same (× 50); C, D, vertical sections of small, distorted pycnidia formed in the abaxial ridge tissue of the leaf (× 150); E, vertical section of large spherical pycnidium (× 50); F, part of outer wall of pycnidium (× 555.)

of the developing pycnidia is prevented. However, the pycnidia are not elongated parallel to the vascular strands as pycnidia of other fungi often are on leaves with the veins close together.

The pycnidial wall is complete, 14–40 μ thick and composed of several layers of flattened cells. The cells of the outer layer are angular, irregular in size, 2–10 μ across and have clearly defined dark brown walls (Fig. 3F). By transmitted light under low power of the microscope the pycnidial walls appear Light Brownish Olive to Brownish Olive.

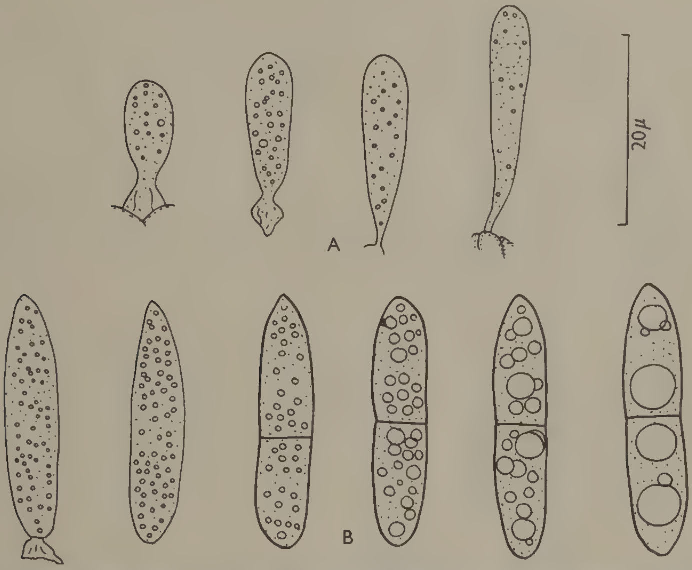

Fig. 4. *Ascochyta typhoidearum.* A, young attached spores in various stages of development; B, series showing subsequent changes in detached spores within the pycnidium. (Both × 1250.)

Spores in various stages of development can be seen attached to the inner cells of the walls of pycnidia crushed in aqueous methylene blue (Fig. 4A). The proximal end is attenuated and the spore often becomes detached at this point when it is about half-size and before the septum is formed. Young spores free within the pycnidium have many small oil drops and soon develop a single median septum (Fig. 4B). The mature spores are straight, fusoid with more or less pointed ends, and have colourless smooth walls (Fig. 5A). There are usually two large oil drops in each cell and a few smaller ones. Older spores show further coalescence of the oil which forms four large drops in each (Fig. 4B). The mature spores are often slightly constricted at the septum, a feature accentuated by mounting in water which is taken up by the cells and causes them to bulge laterally. The spores have been reported as eventually becoming 3-septate (Grove, 1936), but though this fungus has been found and examined many times in the present investigation only one spore with more than one septum has been seen and this had only two (Fig. 5B).

There has been considerable confusion over the naming of this fungus because of a superficial similarity of its spores to those of *Stagonospora typhae* Höhnel, which is commonly found on *Typha* spp. I have no doubt, and the findings of von Höhnel (1909) confirm this point, that this species on *Sparganium* is the same as the fungus on the type material of *Hendersonia typhoidearum* Desm. in the Kew Herbarium. In his original description

Dimensions of 100 spores taken at random from three different collections

Length (μ)			Width (μ)	
Range	Mean	Standard deviation	Range	Mean
25–38	28·6	2·19	5–7	6·0
23–37	28·1	3·06	4·5–7	5·5
21–30	25·9	1·93	4·5–7	6·0

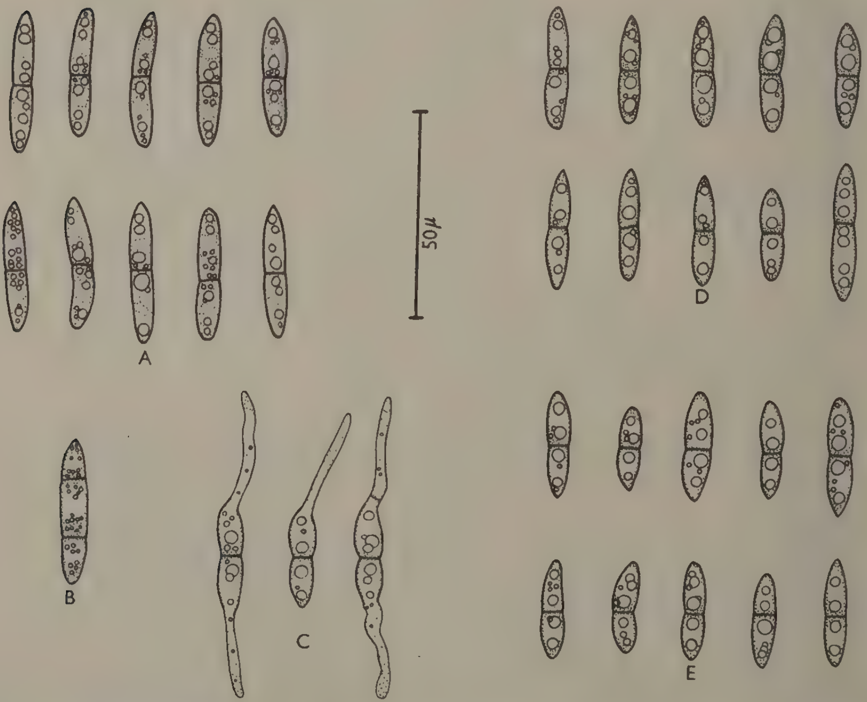

Fig. 5. *Ascochyta typhoidearum*. A, ten random living spores from nature; B, abnormal 2-septate spore from nature; C, germinating spores after 20 hr. in water at room temperature; D, ten random living spores from pycnidia formed on oatmeal agar; E, ten random living spores from pycnidia formed on re-infected sterilized *Sparganium* leaf. (All × 555.)

Desmazières (1849) described the spores as oblong, straight, with obtuse ends, subfusiform and with '...sporulis 4, globosis, hyalinis, remotis' ('les sporules paraissent comme des points brillants'). The type description does not mention spore size, but additional notes give the spore measurements as 25–30 × 5 μ. It is not stated whether any septa were seen.

The mature spores of the type material of this species in Kew Herbarium

(Fig. 6A) are fusiform with pointed ends and the positions of the 'sporules', probably large oil drops, can be seen. Each spore also possesses a median septum and is obviously constricted at this point .They measure 18–26 × 5–7 (average of 50, 22·9 × 5·9) μ and are thus somewhat shorter than the living spores I have measured but this is probably due to the shrinkage of the dried material. Further, spore length is very variable in the living specimens I have collected, and my measurements of spores from only two pycnidia are insufficient to illustrate the full range of spore size that could probably be found in the type material. Von Höhnel gave the spore dimensions as 24–30 × 5–7 μ in the smaller pycnidium which he examined from the type material.

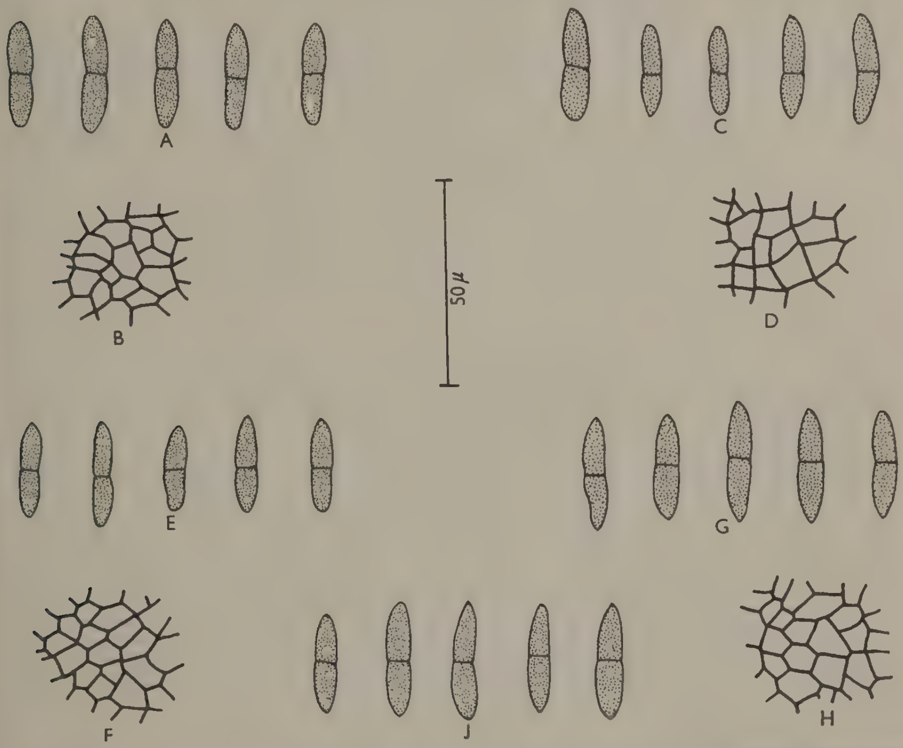

Fig. 6. *Ascochyta typhoidearum.* A, five random spores of type material of *Hendersonia typhoidearum* Desm.; B, part of pycnidial wall of same; C, five random spores of *H. typhoidearum* (on *Sparganium*, Spye Park, Wilts, coll. Berkeley and Broome); D, part of pycnidial wall of same; E, five random spores of type material of *Darluca typhoidearum* B. & Br. v. *sparganii* Fuck.; F, part of pycnidial wall of same; G, five random spores of *Stagonospora typhoidearum* (Desm.) Sacc. v. *sparganii* Fuck. (on *Sparganium*, Meols, Cheshire, coll. J. W. Ellis, 7/2/1914); H, part of pycnidial wall of same; J, five random spores of *Ascochyta quadriguttulata* Kab. & Bub. mounted in lactophenol with cotton-blue (on *S. ramosum*, Llanstadwell, Pemb., coll. P. G. Rhodes, 7/8/1928). (All × 555.)

Desmazières pointed out that his species was part of *Sphaeria duplex* Fr. which consisted of a number of forms on different hosts including *Sparganium*. However, it seems that, at that early date, neither he nor other workers of the same period made a clear distinction between the species on *Typha* and *Sparganium*.

The Berkeley & Broome collections of *Hendersonia typhoidearum* on *Sparganium* in the Kew Herbarium are also of the same fungus. The spores (Fig. 6C) have the typical median septum and measure 20–30 × 4–6·5 (average of 45, 24 × 5·4) μ. Fuckel (1869) was apparently the first to distinguish the fungus on *Sparganium* from the similar one on *Typha* and briefly described *Darluca typhoidearum* (Desm. sub *Hendersonia*) B. & Br. v. *sparganii* Fuck. (= *Phoma sparganii* Fuck.) with spores 26 × 6 μ. I have examined type material of this in Kew Herbarium. The spores (Fig. 6E) measure 18–29 × 4–6 (average of 50, 21·9 × 5·1) μ and, though slightly smaller, are again very similar to those of my material. The median septum and positions of the four large oil drops can be seen in many of them.

Saccardo (1884) transferred *Hendersonia typhoidearum* to *Stagonospora typhoidearum* (Desm.) Sacc. and put Fuckel's variety as a subspecies of this, but Allescher (1901) raised Fuckel's variety to specific rank, citing it as *S. sparganii* (Fuck.) Sacc. However, neither author added anything to the original description and there is no evidence that they examined any material.

With very rare exceptions the spores have only a single median septum and therefore, as von Höhnel (1909) has pointed out, this fungus should be placed in the genus *Ascochyta*. Bubák & Kabát (1910) described *A. quadriguttulata* on leaves of *Sparganium ramosum* from Bohemia. The spores are given as 12–26 × 5–6 μ, cylindrical with a single median septum, sometimes constricted and with two oil drops in each cell. Though no illustrations were published, and I have not seen type material, it seems very likely that this is the same as the fungus dealt with here. Bubák and Kabát emphasized that their species was not the same as *S. sparganii* (Fuck.) Sacc. though this statement was contradicted by Grove (1935). He declared that their fungus is merely a younger stage of *S. sparganii* (Fuck.) Sacc., but I cannot find any evidence to support this. Unamuno (1929) recorded *Ascochyta quadriguttulata* Kab. & Bub. from Spain with spores 1-septate, 17–28 × 4·5–7 μ, generally 4-guttulate, and did not mention the formation of further septa within the spores.

I have examined two relevant collections of British material on *Sparganium* in Kew Herbarium. These are labelled *Stagonospora typhoidearum* Sacc. v. *sparganii* Fuck. (coll. J. W. Ellis, Meols, Cheshire, 7/2/1912) and *Ascochyta quadriguttulata* Kab. & Bub. (coll. P. G. Rhodes, Llanstadwell, Pemb. 7/8/1928). The spores (Fig. 6G, J, respectively) of both collections are again identical with those of my fungus and no doubt belong to the same species.

Confirmation of the common identity of the herbarium material on *Sparganium* that I have examined is found in the close similarity of the outer layers of the pycnidial wall. In all of them the wall is Brownish Olive when viewed by transmitted light under the low power of the microscope and is composed of small angular cells with straight, thick, dark brown, anticlinal walls (Fig. 6B, D, F, H).

S. typhoidearum (Desm.) Sacc. f. *santonensis* Brun. (Saccardo, 1899) was described on *S. ramosum* from France with spores 22–28 × 6 μ, at first medianly constricted, 4-guttulate, later 3-septate. I have not examined type or other named material of this form, but the brief description does suggest that it may be the same as the one I am dealing with here, though the later development of three septa in the spores is not typical.

Presumably, in spite of the doubtful identity of the host of the type material, Desmazières's specific epithet is the valid one for this species. Although von Höhnel pointed out that Desmazières's type material is an *Ascochyta* he did not formally transfer the species to that genus. This has now been done and the synonyms are listed as follows:

1849	*Hendersonia typhoidearum* Desm.
1860	*Darluca typhoidearum* B. & Br. (in part).
1869	*D. typhoidearum* B. & Br. var. *sparganii* Fuck.
1869	*Phoma sparganii* Fuck.
1884	*Stagonospora typhoidearum* (Desm.) Sacc.
1884	*S. typhoidearum* (Desm.) Sacc. ssp. *sparganii* Fuck.
1894	*S. typhoidearum* (Desm.) Sacc. f. *santonensis* Brun. (?)
1901	*S. sparganii* (Fuck.) Sacc.
1910	*Ascochyta quadriguttulata* Kab. & Bub.

Ellis (1914) described *Ascochyta sparganii* on leaves of *Sparganium ramosum* with minute pycnidia up to 40 μ diam. containing hyaline, uniseptate, oblong spores with rounded ends and measuring 16–18 × 6–7 μ. The type material in the Kew Herbarium (Rodborough Common, Surrey, 25/9/1913) which I have examined consists of a few green distal parts of leaves which probably do not belong to *Sparganium*. The pycnidia are grouped on yellowish blotches but I cannot find any spores. In view of the nature and condition of the host material and the minute size of the pycnidia it is very improbable that this is the same fungus as the one I have been discussing here in spite of the fact that Grove (1935) puts Ellis's species together with *A. quadriguttulata* Kab. & Bub. as synonyms of *Stagonospora sparganii* (Fuck.) Sacc.

The spores germinate readily in water at room temperature, a single germ tube usually being formed near the end of each cell (Fig. 5 C). On 2 % malt agar a slow growing colony with an irregular edge is formed. The aerial mycelium is uneven, cottony and uniformly white. The reverse is Dresden Brown centrally but the younger parts are Light Buff grading to an irregular white margin. Colonies on potato-dextrose and oatmeal agars are similar.

On 2 % malt and oatmeal agars the colonies eventually reach the edge of the Petri dish. The aerial mycelium of these old cultures is uneven, cottony, with irregular patches of white to Castor Grey. The reverse is patchy, Hathi Grey to black with areas of Dresden Brown and Light Buff. Growth is much slower on potato-dextrose agar and the edge of the plate is not reached before the medium dries. The aerial mycelium is uneven, felty, and white to Pearl Grey. The reverse is mostly Light Buff but Dresden Brown centrally and at the edges.

Pycnidia are rarely formed on 2 % malt agar but are numerous on both oatmeal and potato-dextrose agars. The spores (Fig. 5 D) are very similar to those found in nature.

On sterilized pieces of *Sparganium* leaf a sparse grey mycelium is formed. The pycnidia, which appear after 3–4 weeks, are mostly on the submerged pieces of leaf though some occur on the upper, drier parts. They are only half immersed in the tissues of the leaf but contain many typical spores (Fig. 5E). Fruiting is also abundant on sterile pieces of *Carex riparia* leaf but here the pycnidia, which also produce typical spores, are found mostly within the tissues of the substratum.

Dimensions of 100 random spores from three different cultures

	Length (μ)			Width (μ)	
Source	Range	Mean	Standard deviation	Range	Mean
Oatmeal agar	22–34	26·6	2·19	4–7	5·6
Re-infected *Sparganium*	20–27	23·0	1·42	5–7	5·9
Re-infected *Carex*	20–27	22·8	1·42	5–7·5	5·9

Material of this fungus has been deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 75294) and the Herbarium, Royal Botanic Gardens, Kew.

I wish to express my gratitude to Prof. C. T. Ingold for his interest and guidance throughout the course of this work. My thanks are due also to Dr R. W. G. Dennis for taxonomic advice.

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JELLY AS A WATER-RESERVE IN FUNGI

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(With 2 Text-figures)

In *Bulgaria inquinans* spore discharge continues even when the drying gelatinous apothecium has lost four-fifths of its original weight. In *Phallus impudicus* evidence is given that the rapid elongation of the stalk involves a considerable increase in weight. In both these fungi the fruit-body is richly supplied with jelly. It is suggested that this represents a water reserve used for spore discharge in the first species and for stipe elongation in the other.

INTRODUCTION

Since generally fungi appear to have weak powers of conduction, the accumulation of a local reservoir of water to maintain the turgidity of the elements of an hymenium or to supply a rapidly enlarging structure may be important. In this paper the hypothesis is advanced that where a relatively large amount of jelly is to be found in a fungal structure, it represents an immediately available water reserve. Two concrete examples are considered in this connexion: the apothecium of *Bulgaria inquinans* and the sporophore of *Phallus impudicus*.

SPORE DISCHARGE IN *BULGARIA*

The black, rubbery apothecia of this common gelatinous discomycete are found on the bark of dead trunks of broad-leaved trees (e.g. beech and oak) in autumn.

A number of apparently fully imbibed apothecia were collected and brought into the laboratory. Each apothecium (or sometimes a group of two or three closely adhering specimens) was suspended by a nylon thread, passed through the stalk region, from a glass rod resting on the rim of a 400 ml. beaker so that the apothecium hung about half-way down the beaker with the hymenium facing the bottom. This set-up was placed in a dry, glass-fronted cupboard and subjected to the ordinary rhythm of natural illumination. The temperature was fairly constant (20 ± 1 °C.) and conditions of evaporation, as measured by an evaporimeter, remained extremely constant during the course of the experiments.

Discharged ascospores collected at the bottom of the beaker which was changed each morning (*c.* 10 a.m.) and each evening (*c.* 6 p.m.) Their number was estimated by suspending them in a definite volume of water and counting with a haemacytometer. From these determinations the average hourly output of spores for each day period and for each night period could be calculated. In making the counts only the large pigmented ascospores were considered, the small colourless ones being ignored. In

Bulgaria inquinans the ascus normally contains four large dark spores and four small colourless ones. Each morning and evening, when the beakers were changed, the apothecia were weighed. The results of two experiments are shown in Figs. 1 and 2.

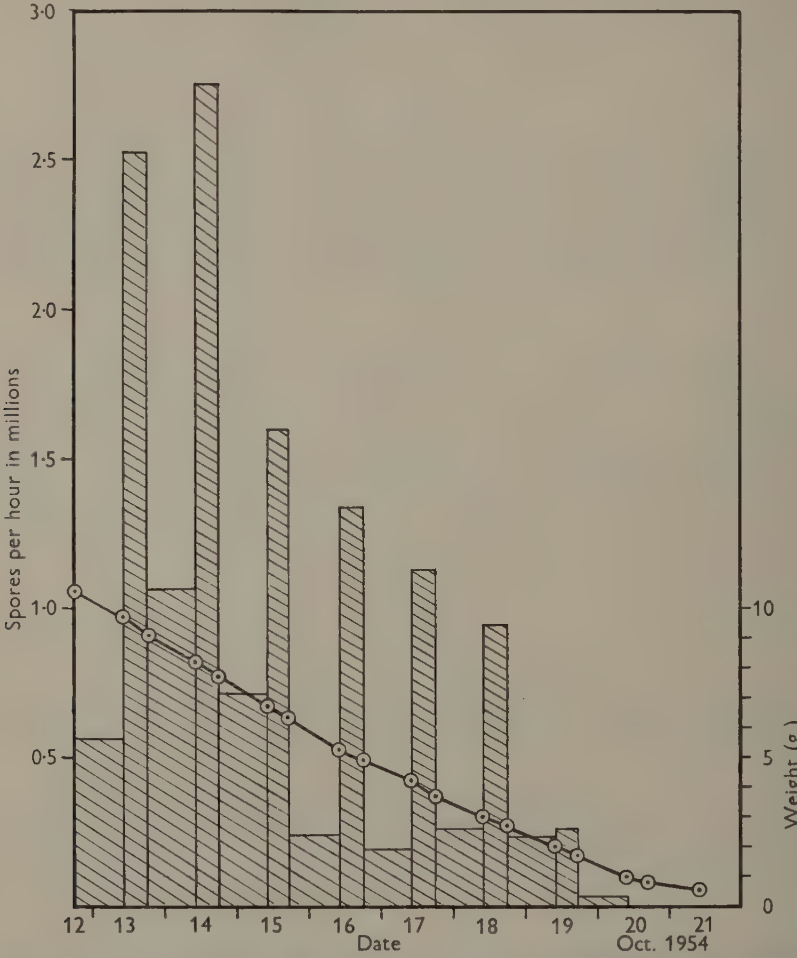

Fig. 1. Spore liberation from a single drying apothecium of *Bulgaria*. The continuous line refers to weight plotted against time. The histograms show average hourly rate of spore liberation for day periods (10 a.m.–6 p.m.) and for night periods (6 p.m.–10 a.m.).

First it is clear that discharge tended to be diurnal, the average rate of spore liberation being much greater in the day periods than in the periods (6 p.m.–10 a.m.) dominated by many hours of darkness. Secondly, as the apothecia dried the rate of discharge, allowing for the diurnal rhythm, tended gradually to fall. However, in both the experiments (Figs. 1, 2) reported here and in two further experiments, details of which are not given, spore discharge continued until the apothecia were reduced to less than a fifth of their original weight and appeared very shrivelled. After discharge finally ceased, a slight further loss of weight occurred before the apothecia came into equilibrium with the humidity of the surrounding air. Since discharging asci are essentially turgid cells, it is clear that in the shrivelled apothecia in which discharge still continues, water remains available and is presumably being extracted from the jelly.

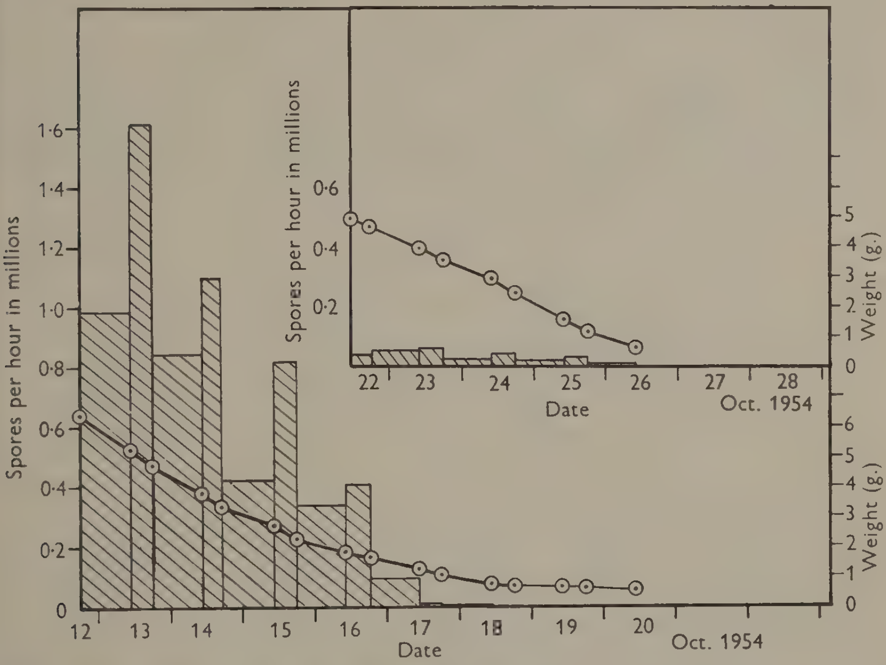

Fig. 2. Spore liberation from a small group of drying apothecia of *Bulgaria*. Main figure shows behaviour during first drying with continuous line giving weight plotted against time. The histogram shows average hourly rate of spore liberation for day periods (10 a.m.–6 p.m.) and for night periods (6 p.m.–10 a.m.). Inset figure shows behaviour following rewetting.

If shortly after an apothecium has ceased to liberate spores as the result of drying, it is allowed fully to re-imbibe either by soaking in a dish of water or by being placed under a dripping tap, it never regains its full original weight but only 70–80 % of it. Further, although spore discharge may occur from such re-imbibed specimens, the rate is invariably low (Fig. 2) and sometimes no measurable output occurs.

STIPE ELONGATION IN *PHALLUS*

In the 'egg' stage of *Phallus impudicus*, just before the soft 'shell' is ruptured, the crisp spongy stipe bearing the spore-covered cap is surrounded by a three-layered peridium. The outer and inner layers of this are thin and papery, but the middle one consists of jelly, which contributes the greater part of the weight of the whole structure. This jelly is composed largely of water. The 'egg' is attached to a rhizomorph which, when traced backwards, is finally found to be attached to buried wood, but the connexion may be several feet away. Presumably the material, including water, accumulated in the 'egg' has been gradually translocated from this food base through the rhizomorph. It seems likely that the biological significance of the large amount of jelly in the 'egg' is connected with water supply permitting, at the appropriate time, the elongation of the stipe which occupies only a very few hours. To test this hypothesis it is necessary to show that stipe growth does involve increase in weight.

An opportunity to study this problem came during the Autumn Foray at Sheffield in 1956. In a coniferous wood close to Chatsworth House, in Derbyshire, stinkhorns occurred in considerable numbers. In all fruit-bodies (a total of six) gathered, the stalk was expanded but the spore-containing slime was still completely intact. From each of these the stipe was separated and placed in a polythene bag to prevent loss by evaporation. In *Phallus* the stipe is a very definite morphological entity readily and cleanly separable from the rest of the fruit-body. In addition the first six 'eggs', judged to be essentially mature and almost ready to burst, were gathered. A few hours later in the laboratory the stipes taken from the expanded sporophores were weighed and the stipes from the 'eggs' were carefully dissected out and also weighed. The data are given in Table 1. It will be seen that the mean weight of the elongated stipes is more than double that of the stipes from the unexpanded sporophores. Although the samples are small and each is rather variable the difference of the means is highly significant ($P < 0{\cdot}01$).

Table 1. *Increase in weight of stipe in* Phallus impudicus *after elongation*

	Wt. of stipes (g.) from freshly expanded sporophores	Wt. of stipes (g.) from mature unexpanded eggs
	17·1	11·1
	20·7	6·8
	7·6	6·6
	12·0	3·1
	15·5	2·5
	10·3	4·4
Mean	13·8	5·8

The results are thus fully consistent with the hypothesis that the water in the jelly is used as a local water reservoir for stipe expansion, although there is no proof that the jelly is reduced in weight and loses water as the stipe expands.

My thanks are due to Vivienne Dring for conducting the experiments using *Bulgaria* and to Susan Hadland for the preparation of the figures.

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AQUATIC SPORA OF OMO FOREST, NIGERIA

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(With 6 Text-figures)

An account is given of spores from scum and foam collected from certain Nigerian streams. Two distinct types of spore belonging to *Campylospora* spp. are recognized. A kind of spore closely resembling that of *Speiropsis pedatospora* Tubaki is described. The presence of spores of an undescribed *Heliscus* sp., previously reported from East and Central Africa, is noted. Spores of *Robillarda* sp. were seen in one scum sample. A tetra-radiate septate spore with clamp connexions is illustrated.

Attention has been drawn (Ingold, 1956, 1958; Dixon, 1959) to the aquatic spora of streams in various parts of Africa. The general range of form of fungal spores found in samples of scum and foam from small streams flowing rapidly through wooded regions is very like that seen in similar collections from Britain. Nevertheless, although some of the spores are clearly of species known outside Africa, a number appear to belong to species not yet described.

So far as Nigeria is concerned I had previously studied the spora of a small stream very close to the Botany Department of University College, Ibadan (Ingold, 1956). On this second visit (June 1958) spores from another stream (Ona River) bordering the Botanic Gardens about a mile from the Department, and also from two small streams in Gambari Forest some 10 miles south-east of Ibadan were examined. All the spore types seen on the previous visit in the stream close to the Department were again observed in these collections, but no new ones were noted.

Scum was then collected from three streams in the Omo Forest about 70 miles south-east of Ibadan. In the scum, although some spore types were common to the spora observed close to Ibadan, other types were not. Amongst the former were frequent spores of *Triscelophorus monosporus* and *Actinospora megalospora*, both British species. Very common in the Omo scum were spores clearly belonging to two species of *Campylospora* (Fig. 1 A, B). The larger type agrees completely with the description and figures of *C. chaetocladia* (Ranzoni, 1953). However, the smaller has much more delicate 'hairs' and there are differences of general shape between the two kinds of spore which are, however, rather difficult to define. The spores previously identified (Ingold, 1956) from the Ibadan stream as those of *C. chaetocladia* clearly belong to this second species. The presence of these two species has been reported by Dixon (1959) in Ghana. Before Ranzoni, working in California, had published his account of *Campylospora*, spores (Fig. 1 C) obviously referable to this genus had been figured from Britain (Ingold & Ellis, 1952). These do not seem to me to agree in detail with either of the species from the Omo Forest. It would appear, therefore, that there are at least three species of *Campylospora*, although formal description of the other two must await further knowledge of the fungi. *C. chaetocladia* recorded from East Africa (Ingold, 1958) seems to have been correctly identified.

In a previous paper (Ingold, 1958) the morphology of H-shaped spores with a short pointed branch hanging from the horizontal part of the letter, collected from a Leicestershire stream, was considered and their possible relationship to *Campylospora* was discussed. Three exactly similar spores were seen in the Omo spora (Fig. 2C).

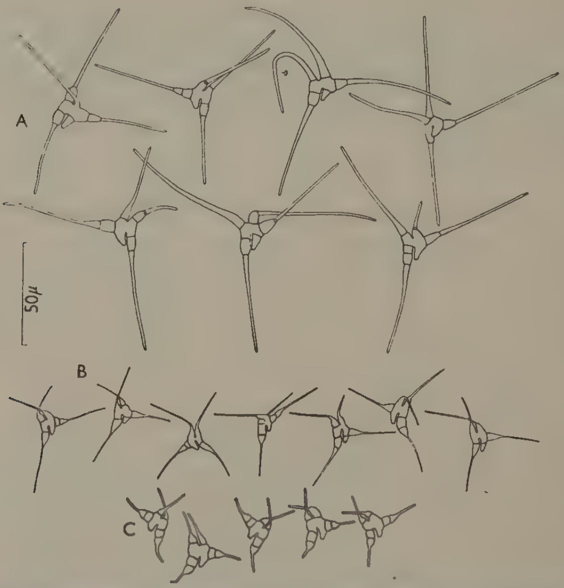

Fig. 1. A, spores of *Campylospora chaetocladia* from Omo scum; B, spores of smaller species of *Campylospora* from Omo; C, spores of *Campylospora* sp. from Wheatfen, England.

A frequent tetra-radiate spore-type is shown in Fig. 3A. This has a narrow, tapered and apparently non-septate stalk-part to the broader end of which three much longer, fatter and obviously septate arms are attached. This spore-type has also been reported from Ghana (Dixon, 1959). I have a strong impression that this agrees basically with a type figured from Uganda (Ingold, 1958, fig. 1) which, however, had six to eight arms attached to the stalk of the spore. It seems likely that two different species of the same genus are concerned.

A very common spore (Fig. 4), already figured from Ghana (Dixon, 1959), appears to consist of a main axis with two lateral branches, one arising from the third and the other from the fourth segment of the main axis. What particularly distinguishes this type is that the axes consist of chains of sausage-shaped cells separated by constrictions. Further, each cell contains two vacuoles. I had originally thought that this might represent an undescribed species of *Tricladium*. It is clear, however, that the spores show close agreement with those of *Speiropsis pedatospora*

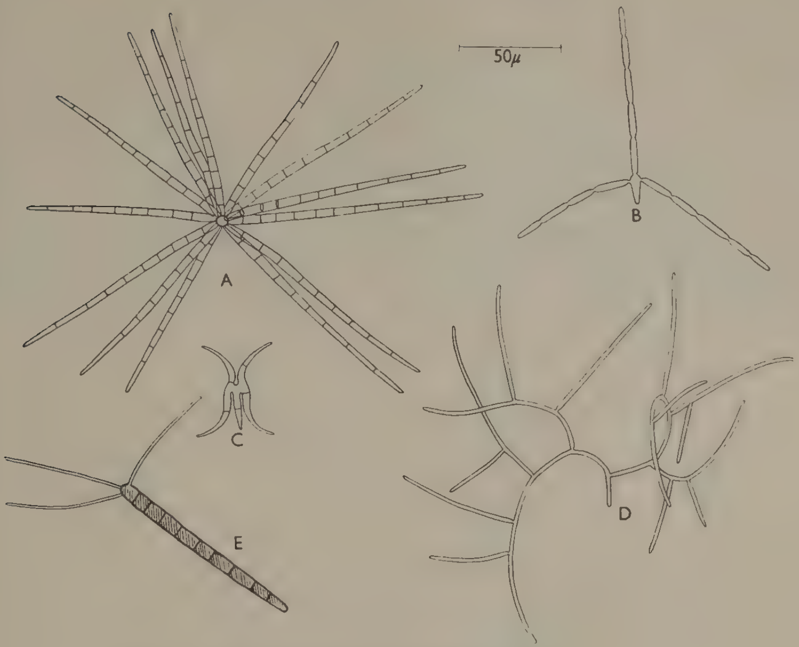

Fig. 2. A–E, various spore types from Omo scum.

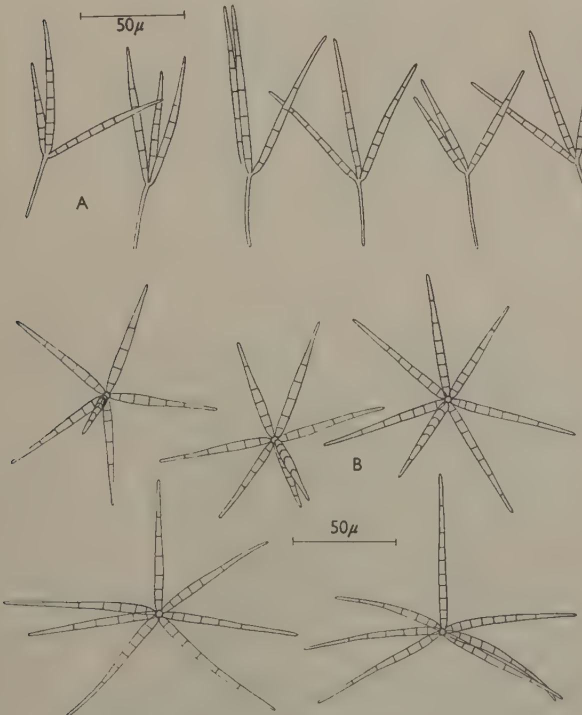

Fig. 3. A, B, two common spore types from Omo scum.

described by Tubaki (1958) as the type of a new genus. It is a terrestrial species found on leaves of *Eriobotrya japonica.* Most of the spores of this fungus figured by Tubaki consisted of a main axis with two laterals, one arising from the basal cell of the main axis and the other from the cell above. Were it not for the consistent difference in the position of branching, I should have been inclined to refer the spores from Omo to Tubaki's species. Certainly, if his new genus is justified, the Omo spores should find a place there, particularly as in both the Japanese and African spores each sausage-shaped cell has the same internal structure with twin vacuoles. However, it is arguable that Tubaki might have placed his fungus in *Tricladium.*

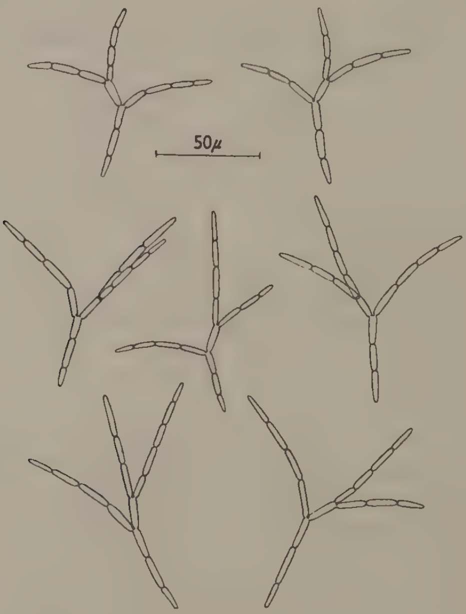

Fig. 4. Common spore type in Omo scum.

Another type of spore, only three of which were seen, was one previously reported from Namulonge in Uganda (Ingold, 1958, fig. 3C). It is tri-radiate and each arm is strongly constricted at intervals, probably representing the positions of cross-walls (Fig. 2B).

I have seen, on scum samples from East Africa, long, septate, sigmoid spores constricted at each septum and with the curvature not confined to a single plane. It seems to me possible that these three fungi with spores having sausage-shaped cells, in spite of differences in the form of the spore, may represent a 'natural' assemblage of species.

One of the commonest spores, of which hundreds were examined, consists of seven (rarely six or eight) long, tapering arms attached to a minute central, spherical region (Fig. 3B). Dixon (1959) has illustrated a spore of this kind from Ghana. Another, but much rarer type, probably cogeneric with the 7-radiate form, consists of about sixteen arms (Fig. 2A). It appears to correspond to the multi-radiate type reported earlier from Ibadan (Ingold, 1956) and from Ghana (Dixon, 1959). One type of spore (Fig. 2D), which was not common, appeared to belong to an undescribed species of *Varicosporium*. The spores agree with those of *V. elodeae* in being branch-systems of varying extent but of the same basic pattern, namely a primary axis developing several secondary axes, usually all on one side, which again may branch to form tertiary axes. As in *V. elodeae* each lateral is constricted at its insertion. The differences lie in the greater degree to which the branch system may be developed and particularly in the tendency for the main axis and at least the secondary laterals to be strongly curved.

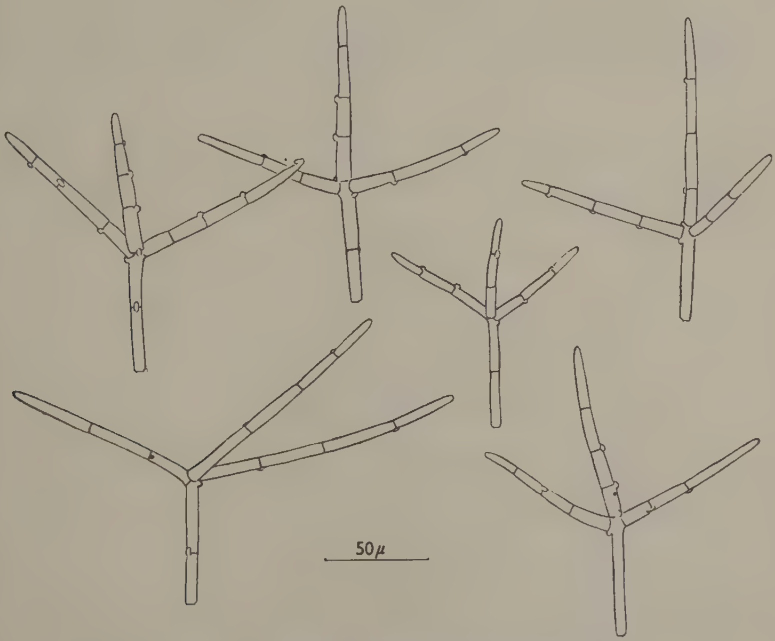

Fig. 5. Spores with clamp connexions from scum samples 15 and 25 miles from Ijebu-Ode on way to Omo.

In the Omo spora a single tetra-radiate septate spore was observed which appeared to have clamp connexions. In two further scum samples collected for me in December 1958 by Mr C. N. Williams from streams, one 15 and the other 25 miles from Ijebu-Ode on the way to Omo, this kind of spore was quite frequent and could be thoroughly studied. The spore (Fig. 5), which is invariably tetra-radiate, is large, hyaline and appears to consist of a main axis bent somewhere near its middle where two lateral arms arise. In this respect its general structure would seem to agree with spores in the genera *Tetrachaetum* and *Alatospora*. The spore had obviously been attached to its parent mycelium by the tip of one of its four arms and this arm is clearly identifiable first by its being generally shorter, secondly by the truncate nature of its end as compared with the tapered apices of the other three, and thirdly by the arrangement of the clamp connexions. When a spore is examined, clamp connexions can be seen at a number of septa and at the others their apparent absence is because they are hidden away on the under-side. They cannot be focused through the hyphae because of the denseness of the protoplasmic contents. However, by rolling the spore about under the coverslip, clamp connexions associated with all septa can often be demonstrated. Presumably in the developing spore there were three growing points, namely at the end of the main axis and at the ends of the two laterals. In two spores growing tips were seen in the early stage of clamp formation.

The occurrence of these spores with clamp connexions in stream scum is of great interest in the whole context of the story of the tetra-radiate spore. It strongly suggests that an aquatic basidiomycete is involved, reproducing by a method of accessory asexual reproduction exactly parallel with the aquatic hyphomycetes. Truly aquatic basidiomycetes are so far unknown and it is, therefore, greatly to be hoped that further work involving the isolation of this fungus and its study in the living state can be undertaken. If it should be fully established that these spores with clamp connexions belong to an aquatic basidiomycete, it would still further reinforce the already well-attested story of the parallel evolution of this type of propagule in the aquatic environment.

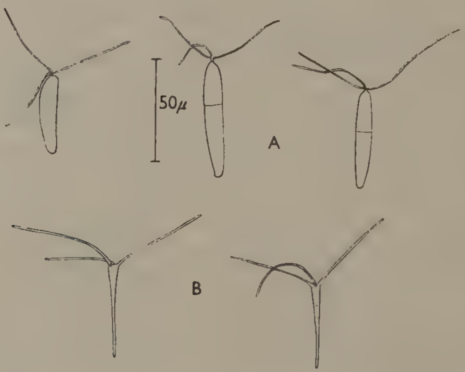

Fig. 6. Spores from scum samples 25 miles from Ijebu-Ode on way to Omo.

Two other kinds of spore from the collections made on the way to Omo merit attention. Thus, the spore-type (Fig. 6B) believed to belong to a species of *Heliscus* and figured (Ingold, 1958) both from Uganda and Southern Rhodesia was very abundant in one of the scum samples. This also contained not infrequent hyaline spores (Fig. 6A) certainly belonging to *Robillarda*, a genus of Sphaeropsidales (Cunnell, 1958). It is interesting that in the pycnidial Fungi Imperfecti the tetra-radiate spore has also been evolved presumably in relation to the aquatic habitat.

A type of spore seen on two or three occasions in the Omo scum consisted of a long, clearly septate part with relatively thick cell-walls impregnated with a brown pigment (Fig. 2E). At one end three long, colourless, unseptate, hair-like outgrowths occurred. Dixon (1959), who found the same kind of spore in Ghana, suggests that it may belong to *Pestalozzia*. In spite of its tetra-radiate form, I think it may not be a spore of an aquatic fungus. In my experience the cell walls of aquatic conidial fungi are thinner and essentially hyaline. It must be emphasized that tetra-radiate spores are not invariably aquatic, and that spores in the foam and scum of streams may be derived from the air as well as from fungi submerged in the running water. An example is the tetra-radiate spore of the terrestrial fungus *Tetraploa aristata*, occasionally seen in scum samples from British streams though vastly outnumbered by truly aquatic tetra-radiate spores.

As in other samples of aquatic spora there are many spores of the unbranched worm-like type, often sigmoid and with curvature frequently in more than one plane. These have not been considered here, largely because their relative simplicity of form makes identification very precarious. However, one type very abundant in the scum almost certainly belonged to *Flagellospora curvula*,

A striking feature of the Omo spora was its close agreement with that described by Dixon (1959) from Ghana, so much so that it was difficult to believe that the two sets of samples had been collected in different territories.

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A STUDY OF SOME ISOLATES OF *PILOBOLUS*

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(With 2 Text-figures)

Twenty single-sporangium isolates of *Pilobolus* from various sources grown in pure culture under standard conditions were studied morphologically. An attempt was made to refer them to described species. Spore size and columella shape appear to be the chief characters of taxonomic value. A precise definition of species must await the study of a much larger range of isolates.

INTRODUCTION

The aim of this work was to determine how far single-sporangium isolates of *Pilobolus* from different sources fitted into recognized species of the genus. Isolates were obtained from dung of various herbivores (horse, cow, sheep and rabbit), each isolate being started from a single discharged sporangium and maintained in pure culture on Page's dung-extract agar (Page, 1952), substituting horse for cow dung. For detailed morphological study each isolate was subcultured on to sterilized horse dung and grown at 13 ± 1° C. in light from 'daylight' fluorescent tubes. A sufficient quantity of horse dung was obtained at the outset, air-dried, mixed and stored. This stock of material was used for all sub-cultures, thus maintaining reasonable constancy of medium. The diurnal rhythm of sporangial ripening was broken by keeping the cultures in continuous light, so that sporangiophores at all stages of development were available at one time for study (McVickar, 1942; Klein, 1948).

SPORE SIZE AND SHAPE

As the size and shape of spores have figured so prominently in the taxonomy of *Pilobolus*, these features were considered with special care. Because it was thought that spore size might alter after discharge, measurements were made only of spores (100 from each) from sporangia discharged within the previous 24 hr. Altogether twenty isolates were studied. On the basis of spore size and shape these could be divided into five groups (Fig. 1):

A. Spores elliptical and relatively small ($4·5–8·5 \times 3·0–5·5\,\mu$).
B. Spores elliptical and relatively large ($10–16 \times 6–11\,\mu$).
C. Spores intermediate in size between those in A and B, and more broadly elliptical than in B ($8–13·5 \times 6–9·5\,\mu$).
D. Spores subspherical ($10·5–13·5 \times 9·5–12·5\,\mu$).
E. Spores spherical and of variable diameter ($7·5–14·5\,\mu$).

Details of spore sizes of the 20 isolates are given in Table 1.

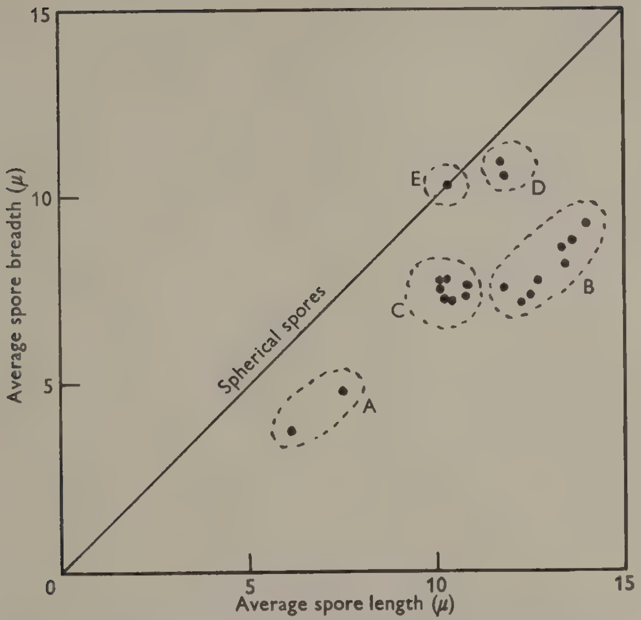

Fig. 1. Average spore dimensions of twenty isolates of *Pilobolus*. Types with spherical spores lie on the diagonal line.

Table 1. *Spore dimensions of twenty* Pilobolus *isolates*

Isolate no.	No. of sporangia used	Total no. of spores measured	Mean dimensions (μ)	Range (μ)
A1	3	300	6·1 × 3·7	4·5–8·5 × 3·0–5·5
A2	2	200	7·5 × 4·7	6·5–8·5 × 3·5–5·0
B1	10	1000	13·3 × 8·6	11·0–16·0 × 7·0–11·0
B2	13	1300	13·6 × 8·8	11·0–16·5 × 7·0–11·0
B3	2	200	12·2 × 7·1	10·5–14·0 × 6·0–8·0
B4	2	200	12·7 × 7·7	11·0–14·5 × 7·0–9·5
B5	3	300	11·8 × 7·5	10·0–14·5 × 6·0–9·0
B6	3	300	12·5 × 7·3	10·5–15·0 × 6·0–9·0
B7	3	300	13·4 × 8·1	11·0–16·0 × 6·5–9·5
B8	3	300	14·0 × 9·2	12·0–16·0 × 8·0–11·0
C1	16	1600	10·9 × 7·6	9·0–13·0 × 6·0–9·0
C2	3	300	10·1 × 7·7	8·0–12·5 × 6·0–9·5
C3	3	300	10·3 × 7·2	9·0–12·0 × 6·0–8·5
C4	3	300	10·1 × 7·5	9·0–12·0 × 6·0–9·5
C5	2	200	10·2 × 7·3	8·5–13·5 × 6·0–9·5
C6	2	200	10·8 × 7·3	9·0–12·5 × 6·5–9·0
C7	4	400	10·2 × 7·7	9·0–12·0 × 6·5–9·5
D1	2	200	11·8 × 10·5	10·5–13·5 × 9·5–12·5
D2	1	100	11·7 × 10·8	10·5–13·0 × 9·5–12·0
E1	4	400	10·3 diam.	7·5–14·5 diam.

OTHER CHARACTERS OF POSSIBLE TAXONOMIC VALUE

Columella. The columella in group A is a low-pitched or flatly convex dome in the intact sporangiophore. After discharge the edge turns inwards as in a beret. In the other groups (B–E) the columella is somewhat teat-like, and in the discharged sporangium is distinctly hat-shaped (Fig. 2). It seems that the shape of the columella may be of some taxonomic value, since in my isolates these two types of columella are substantially distinct.

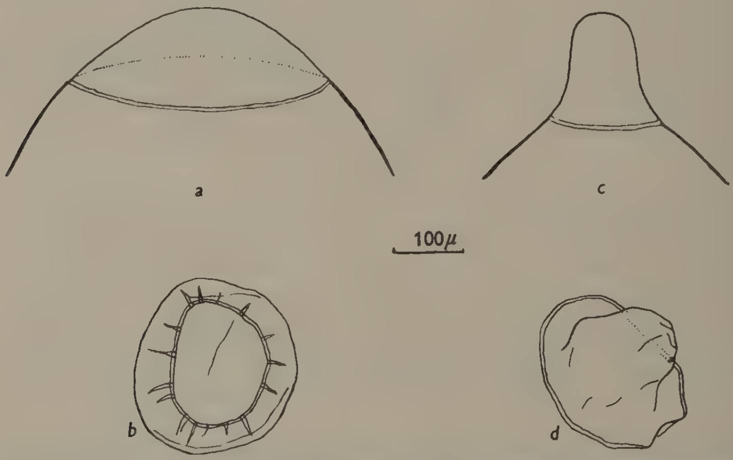

Fig. 2. Columella type in *Pilobolus*. *a* and *b* from a type A isolate: *a*, columella attached to sporangiophore; *b*, columella, seen from below, dissected from a discharged sporangium. *c* and *d* from a type B isolate: *c*, columella, attached to sporangiophore; *d*, columella, seen from above, dissected from discharged sporangium.

Trophocyst. Grove (Buller, 1934) described the trophocyst as mainly terminal in some species but intercalary in others. In all my isolates the trophocyst appeared to arise in an intercalary position, as described by Buller (1934), although it may finally give the appearance of being terminal. On the basis of my isolates, it does not seem that the position of origin of the trophocyst is of reliable taxonomic value. So far as shape is concerned the trophocyst shows a wide variety of form, but none of my isolates has consistently produced the very long trophocyst which is supposed to be typical of *P. longipes*.

The close clustering of trophocysts has been commented upon by Buller (1934) for *P. roridus* and *P. nanus*, and has been used by Naumov (1939) to separate species. Isolate A 1 was the only one which has shown this character, and then only whilst it was in a vigorous condition. After a period in culture the feature seems to be lost.

Stature. Sporangiophore height and the sporangial diameter do not appear to be useful characters in grouping my isolates. In any one single-sporangium culture these characters vary considerably. Furthermore, Klein (1948) found that the heights of sporangiophores varied with light intensity.

Consistency of jelly in the sporangium. How the spores spread out when mounted in water appears to be determined by the consistency of the jelly accompanying them in the sporangium. Thus in the single isolate placed in group E the spores disperse very readily in water as compared with all the others. This feature may possibly have some value in characterizing species.

DISCUSSION

The question arises as to how these isolates should be identified. The fullest modern treatments of the taxonomy of *Pilobolus* are those of Grove in Buller's *Researches on Fungi* (1934) and Zycha (1935); Naumov (1939) has also produced a key. Having regard to spore dimensions, the two isolates of group A seem to agree with *P. crystallinus* except that the spores are slightly smaller than Grove suggests. The eight isolates of group B seem to agree with *P. kleinii*. However, the dimensions of the spores of group C (seven isolates) do not closely agree with the dimensions of any of the previously well-described species, though they approach most nearly to *P. kleinii*. The spores of the two isolates in group D are subspherical and relatively thick-walled, but the wall appears to be composed of a single layer not two as described for *P. oedipus*. The spore dimensions approach those of *P. longipes* but the isolates did not resemble that species in the other specific characters, namely the very much larger size of the sporangiophore and the elongated trophocyst.

The spherical spores of the single isolate placed in group E are relatively variable in diameter, but smaller than those of *P. sphaerosporus*, the only clearly described species with thin-walled, spherical spores (Table 2).

Table 2. *Spore dimensions of some described species*

(Data from W. B. Grove in Buller's *Researches on Fungi*, **6**, 190–224)

Species	Range of spore size (μ)
P. crystallinus	5–10 long: [8–10 × 5–6, van T.; 5–12 × 3–6, Palla; 6 × 4·7, Brefeld; 7–11 × 4·5–7, Duvevre]
P. roridus	6–8 × 3–4 (van T.)
P. umbonatus	5–6 × 3–3·8
P. rongipes	12–15 × 10–12
P. kleinii	11–20 × 6–10
P. heterosporus	8–20 (or 25) × 6–12
P. sphaerosporus	Spherical, 10–20 diam.
P. oedipus	Spherical, 9–16 diam. (2-layered wall)

Grove considered that *P. heterosporus* and *P. sphaerosporus* are probably mere varieties of *P. kleinii*. If this view is accepted, a polymorphic species with elliptical or spherical spores is involved and all the isolates in groups B, C and E could be allocated to this species. In my work no culture with both elliptical and spherical spores, and therefore corresponding to *P. heterosporus*, has been found.

With regard to columella shape, the two isolates in Group A differ from all the others in having a low dome-shaped columella. According to the illustrations in Grove's account, *P. roridus*, which may be a variety of *P. crystallinus*, and *P. umbonatus* have this type of columella, which he describes as bluntly conical or rounded. The typical *P. crystallinus* is described as having a conical columella.

As a result of this study it appears to me that the only consistent characters which would seem to be useful in separating species are spore size and columella shape, although it must be remembered that probably only a small number of species is represented among my isolates. An examination of the taxonomic literature on *Pilobolus* and of actual specimens would suggest that species have been based on relatively insignificant and variable characters. As in other genera of 'moulds', such as *Penicillium* and *Aspergillus*, the taxonomy of the genus can probably be placed on a sound basis only after the study of a great many isolates from many sources. The recognition of existing species would seem to be justified only if constant morphological features are involved, such as columella shape (*P. roridus* and *P. umbonatus*) or sporangium form (*P. umbonatus*). How far in fact spore shape and size can really be useful in discriminating between species must await the study of large numbers of isolates.

My thanks are due to Prof. Ingold for his help and advice throughout this investigation.

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SOME FACTORS AFFECTING GROWTH OF *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY

I. *P. INFESTANS* ON LIVING POTATO LEAVES

By P. H. LOWINGS and ISABEL GARCIA ACHA*
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(With 6 Text-figures)

Field experiments indicated that a preceding leguminous crop, or high inorganic nitrogen nutrition, might, under some conditions, be associated with increased resistance of Majestic potato leaves to attack by *Phytophthora infestans*. Increased resistance was associated with slow growth of the parasitic hyphae after invasion, but there was no accompanying change in rate of necrosis of the invaded tissues. Under glasshouse conditions, hyphal growth was stimulated in the leaves on the upper parts of the haulm after onset of host senescence. Similarly, hyphal growth in excised leaves maintained in water was more rapid than that in leaves remaining on the plant, but this difference was reduced if the plants were already becoming naturally senescent. High nitrogen nutrition of the host may delay the development of extreme susceptibility so long as it delays the onset of senescence. It is also suggested that chemical changes in senescent leaves may influence the nutrition and growth of the parasite independently of any toxins liberated during cell necrosis.

INTRODUCTION

In the late summer of 1956 one of the authors (P. H. L.) noticed striking differences in the development of potato blight on Majestic potato plants growing in experimental plots on the Cambridge University Crop Husbandry Experimental Station. The potatoes had been grown as part of a technique for measuring the fertility of plots previously cropped with various types of ley and subsequently receiving uniform dressings of phosphate and potash (F. Hanley & W. J. Ridgman, unpublished). Where the land had previously carried a rye-grass ley, or where an all-arable rotation had been followed, blight attacked rapidly and severely, but where the potatoes followed lucerne, or to a less extent after clover, blight developed more slowly and total death of the haulm was delayed by 10–14 days. Such a clear case of a difference in host resistance, apparently associated with soil fertility, is of considerable interest as its investigation might lead to the elucidation of some of the physiological problems involved in the parasitism of potato plants by *Phytophthora infestans*. This

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* Work carried out during the tenure of a research grant from the Fundacion Juan March, Madrid, while on leave from the Instituto 'Jaime Ferrán' de Microbiologia C.S.I.C., Madrid.

paper incorporates the results of a series of small experiments carried out with the object of defining some of the main factors involved in the observed changes in resistance.

Preliminary observations

In 1956 an attempt was made to discover the immediate cause of the increased severity of blight in the potatoes grown as part of an all-arable rotation (treatment A) compared with those following a crop of lucerne (treatment B). The plants growing on land which had previously carried a rye-grass ley had already been killed by blight. The lower leaves of the plants in both series were already dead when observations were started, but while the leaflets on the upper half of each plant in treatment A bore large numbers of normally developing blight lesions (Fig. 1*a*) those in treatment B bore only two or three normal lesions. There were, however, many lesions on the latter plants which had ceased to develop, leaving an angular necrotic spot up to 1 cm. across, but sometimes an area of *P. infestans* survived at one margin (Fig. 1*b*). The cessation of hyphal growth in these lesions suggested that the environment inside the leaves was unfavourable for hyphal development (a condition which might also influence the incidence of successful infections); therefore growth of the hyphae was studied after infection had been established.

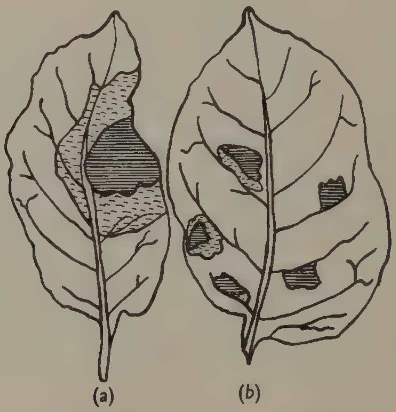

Fig. 1. Lesions caused by *P. infestans* in potato leaflets. (*a*) Typical lesion on plants grown as part of an all-arable rotation. (*b*) Partly arrested lesions common on plants following a lucerne ley. Continuous hatching represents necrosis, broken hatching represents invaded but not yet necrotic areas.

Hyphal growth rates were measured in the field by using, in each treatment, random samples of fifty leaflets, each of which had been naturally infected only at the tip. Each of these leaflets was marked by a numbered tag and for 3 days, using an ordinary ruler, daily measurements were made of the distance from the point where the proximal end of the lamina joined the petiole, to the nearest edge of the visible hyphal front. The distance from the hyphal front to the edge of the advancing necrotic area (see Fig. 1*a*) was also measured. The latter distance was then divided by the daily growth rate of the hyphae to obtain the survival time in days of the leaflet cells after they had been invaded; this will be referred to as the 'necrosis time' (Table 1).

Table 1. *Growth rates of* Phytophthora infestans *hyphae in potato leaves and time taken for host cell necrosis*

	Treatment A	Treatment B	Significance of differences
Mean hyphal growth rate	4·8 mm./day	2·7 mm./day	V. highly significant
Mean distance of hyphal front from necrotic front	6·3 mm.	3·7 mm.	V. highly significant
Mean 'necrosis time' of host cells after invasion	1·30 days	1·37 days	Not significant

Treatment A. Plants grown as part of an all-arable rotation. Treatment B. Plants following a lucerne ley.

In treatment A, then, the hyphal growth rate was higher than in treatment B, yet the necrosis time remained constant; this indicates the presence in the potato leaf of factors influencing hyphal growth independently of cell necrosis. If growth factors were sufficiently low (or inhibitory factors high) while necrosis times remained constant, a condition indistinguishable from hypersensitivity could occur, particularly under climatic conditions adverse to fungal growth. It is thought that this condition could have arisen in the treatment B plants where lesions had ceased to develop normally.

It is common experience that nitrogen nutrition may influence the rapidity with which crops mature, and Müller (1928) and Grainger (1956) are among those who have indicated that early maturity in potatoes may be associated with early blight susceptibility. Grass and straw residues tend to cause temporary removal of available nitrogen from the soil, and leaching may remove nitrogen from land under an all-arable rotation, whereas the slow breakdown of legume residues such as lucerne may release nitrogen at a more steady rate. Field experiments were therefore carried out to determine whether nitrogen alone could influence the susceptibility of Majestic potatoes to blight.

FIELD EXPERIMENTS

A preliminary field experiment was carried out in 1957 but, although it showed just significant effects of high nitrogen nutrition in delaying blight, it was marred by unsuitable weather conditions. The conditions for the confirmatory experiment in 1958 were ideal and this experiment only will be described in detail.

Materials and methods

An area of medium heavy but free draining soil on the Cambridge University Crop Husbandry Experimental Station was chosen for these experiments as it had carried no recent crops. The land was broken in the autumn and cultivated in the spring to obtain a tilth.

The experiment was laid out as a six by six Latin Square, each plot being four rows wide by 12 ft. long. Two guard rows were planted round the complete square. A uniform dressing of 2 cwt./acre of muriate of potash and 3 cwt./acre of superphosphate was applied to the whole area at planting. At the same time one plot in each complete series was rotovated but received no further treatment (treatment 1), while oat chaff at the rate of 3 tons/acre was rotovated into the remaining plots (treatments 2–6) to reduce the initially rather high available nitrogen content of the soil. Plots in some treatments then received Nitra-shell (20·5 % nitrogen as ammonium nitrate) either at planting, or as a later top dressing, or at both times, as shown in Table 2. Ridges were drawn, fertilizers sown and Irish Class A Majestic seed potatoes planted on 20 March; top dressings were applied, where relevant, on 28 May. Ridges were set up at planting and again after the sprouts had emerged.

Table 2. 1958 *field experiment on potato blight*

	Plot treatments			State of plants on 17 July		Days from 17 July to	
Treatment no.	Chaff at planting (tons/acre)	Nitra-shell at planting (cwt./acre)	Nitra-shell top dressing (cwt./acre)	Leaf colour	Flowering stage	50 % blight	75 % blight
1	0	0	0	Yellowing	Flowering nearly over	20·3	26·1
2	3	4	0	Slight yellowing	Flowering nearly over	22·4	28·0
3	3	0	4	Green	Not yet flowering	33·3	38·8
4	3	2	2	Green	In full flower	24·2	29·3
5	3	4	4	Green	In full flower	24·8	30·3
6	3	0	0	Green	In full flower	26·2	31·5

Least significant differences:

50 % blight	3·69 days at 5 % level of significance
	5·00 days at 1 % level of significance
75 % blight	3·59 days at 5 % level of significance
	4·82 days at 1 % level of significance

The plants were allowed to become naturally infected with *P. infestans*. The course of the epidemic in each plot was followed by making regular records of the percentage of leaf area killed, using the blight key issued for survey work by the Plant Pathology Laboratory, Harpenden. Blight development curves for each treatment are illustrated in Fig. 2. The proportions of leaf area infected were transformed by the probit transformation; these transformed proportions were found to bear an approximately linear relation to time. The regression coefficients measuring these relationships were found not to differ between treatments. The fitted regression lines were used to calculate the number of days, from the start of recordings on 17 July, at which 50 % and 75 % leaf area was killed (Table 2). The discrepancy between the relative positions of the curves for treatments 4 and 5 (Fig. 2) and the results for these treatments given in Table 2 is due to the latter being derived from fitted linear regressions, whereas the curves were drawn by joining individual means.

The plants in treatments 1 and 2 came into flower and started leaf yellowing and senescence early, probably due to leaching of nitrogen from the soil by heavy rains. In treatments 3 and 6, where chaff but no nitrogen was added at planting, the shoots were from 10 to 14 days later in emerging than those in the remaining plots, and the development of these plants was still delayed when the blight epidemic started. The plants in treatment 3 were still further retarded as they renewed vegetative growth after receiving their nitrogenous top dressing. The developmental stage reached by the plants on 17 July is summarized in Table 2. It can be seen that the rapidity with which blight attacked the plants corresponded closely with the stage of development reached at the time of the epidemic; the more retarded the plants the slower was the attack. This experiment therefore indicates that the onset of senescence, which may be influenced by nitrogen nutrition, is associated with changes in the susceptibility of Majestic potato plants to blight. Thus, by 10 August both upper and lower leaves had been attacked severely in the senescent plants in treatment 1. In treatment 3, where vigorous growth had been promoted by top dressing previously starved plants, there were few lesions; while in the intermediate treatment 5 the lower leaves, but not the upper, had been severely attacked. These observations agree closely with those made in 1956 on the plants following grass and lucerne leys.

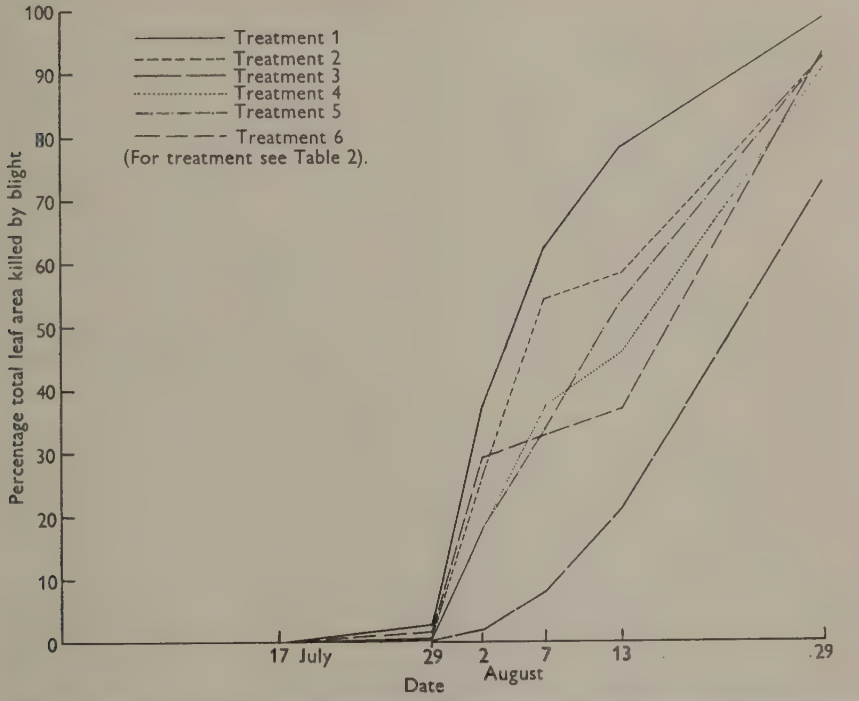

Fig. 2. Blight development curves for plants in field plots subjected to various manurial treatments.

GLASSHOUSE EXPERIMENTS WITH NATURALLY AGEING LEAVES

The field experiments indicated that blight susceptibility was associated with the onset of senescence and appeared to vary according to the position of the leaf on the plant. Glasshouse experiments were therefore carried out to investigate the changes in resistance as each leaf aged.

Materials and methods

To obtain standard plants seed tubers, similar to those used previously and potted up separately in 5 in. flower pots of garden loam, had the sprouts reduced to one on emergence. The tips of the four distal lateral leaflets on each leaf, from the first fully formed basal leaf to the tenth up the plant, were inoculated with zoospore suspensions of *P. infestans*, race 4. Filter-paper confetti was soaked in the suspension and then applied to each leaflet, a further drop of suspension being then added to each piece of filter-paper. After inoculation the plants were kept in polythene-covered humidity chambers, standing in water in a glasshouse. After 4 days the plants were removed from the chambers and kept in a glasshouse cooled and humidified by blowing in finely atomized water; this maintained a very slight water film on every leaf. A light coat of a commercial green shading compound was sprayed onto the outside of the glasshouse to reduce solar radiation.

Batches of plants were sprouted and potted at regular intervals and when a suitable range of plants at different ages was available the leaves were inoculated; growth rates of the hyphae of *P. infestans* were subsequently measured at intervals of 24 hr. by the method described above. As considerable care was taken to maintain all plants in each experiment under standard conditions, the number of plants in each experiment was limited. In the experiment of which the results are discussed below, five plants were used at each of six ages. Planting dates are irrelevant under these conditions, and the plants are best described by characterizing the two youngest series as being in vigorous growth, the next two series as having reached full size but not yet flowering, the penultimate series as being in full flower when inoculated and the oldest series as being post-flowering and bearing yellow lower leaves. In subsequent experiments, fewer series were used but with more plants in each series; the trend of the results was unchanged.

Experimental results

Mean hyphal growth rates in each *leaf* (mean of leaflet figures) at each locus on the plant, from no. 1 at the base to no. 10 nearest the top, are illustrated in Fig. 3. The curves are somewhat irregular because of the relatively small number of plants in each series.

The mean resistance of leaves in the youngest two series of plants was low, the lowest leaves being the most susceptible, while the soft and tender upper leaves were more susceptible than those next below. Once elongation of the haulm had stopped the plants settled down to a steady state, with resistance increasing from the base upwards and with the mean resistance of the plant high. At flowering time there was an indication that the upper leaves again became more susceptible than those in the centre. In the oldest plants the trend of susceptibility was reversed; the very yellow, almost post-mortem lower leaves were highly resistant whereas the upper leaves became more susceptible.

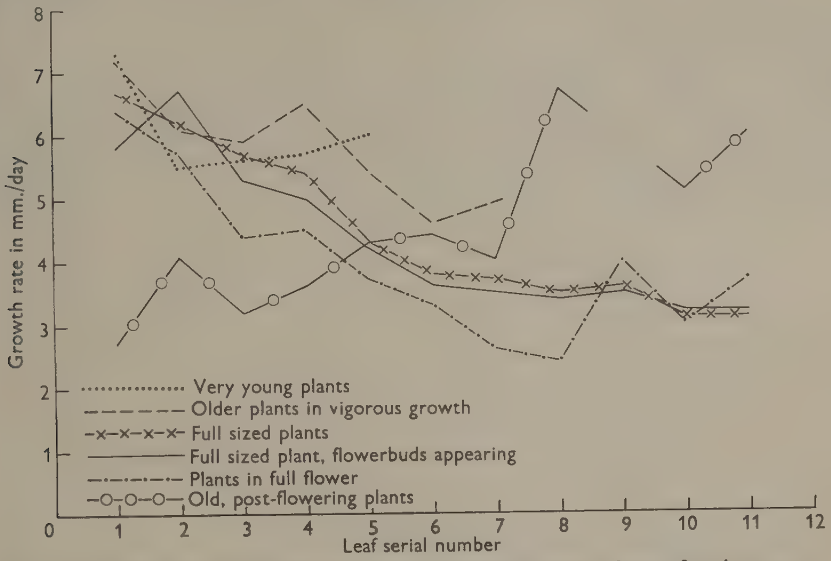

Fig. 3. Growth rates of *P. infestans* hyphae in leaves of potato plants of various ages.

GLASSHOUSE EXPERIMENTS WITH ARTIFICIALLY AGEING LEAVES

Yemm (1956) has suggested that senescence in leaves may be indicated by a predominance of catabolic processes, especially protein break-down. Yellowing in old leaves is accompanied by protein break-down (Michael, 1935), and may be artificially induced in young leaves by carbohydrate starvation in the dark; Gruemmer (1954) has reported increased susceptibility to *P. infestans* of potato plants kept in the dark. Several attempts were made to confirm the latter findings during the course of the present work; although susceptibility to *P. infestans* appeared to increase in potato plants placed in darkness, the susceptibility to *Botrytis cinerea* increased immensely and, because of possible confusion as to origin of lesions, threw some doubt on the interpretation of results. However, most of the biochemical information on protein catabolism in leaves has been obtained by detaching leaves from the plant; Vickery, Pucher, Wakman & Leavenworth (1937) and Yemm (1937, 1950), among others, have shown that protein catabolism consistently starts within a few hours of detaching leaves. The susceptibility of detached potato leaves to *P. infestans* was therefore compared with that of leaves left on growing plants.

Materials and methods

The general technique varied from that used in the previous glasshouse experiments only in the method of obtaining standard plants. Seed potato tubers were sprouted in sand and the sprouts were removed and potted up separately after the first two leaves had appeared. This allowed the use of slightly smaller pots which were more easily handled in the humidity chambers, and it was thought that the removal of the seed tubers would be of little importance in this type of experiment. It was also found possible to select batches of plants which appeared almost identical, and this led to greater consistency in the results.

Series of ten plants were prepared by excising the second and fourth leaves, counting from the first fully formed leaf at the base of the plant upwards; after discarding the proximal pair of lateral leaflets from each excised leaf, the petiole was inserted into a narrow test-tube of distilled water. The test-tubes were then thrust into the soil in the flower pots in which the original plants were growing, each tube being arranged in such a manner that the contained leaf took up approximately the same spatial position that it had occupied before excision. Two days were then allowed for the excised leaves to adapt themselves to the new conditions, and then the leaflets on the excised leaves, and those on the basal, third and fifth leaves, still on the plants, were inoculated; hyphal growth rates were measured as in previous experiments. The distilled water in the test-tubes was topped-up as necessary.

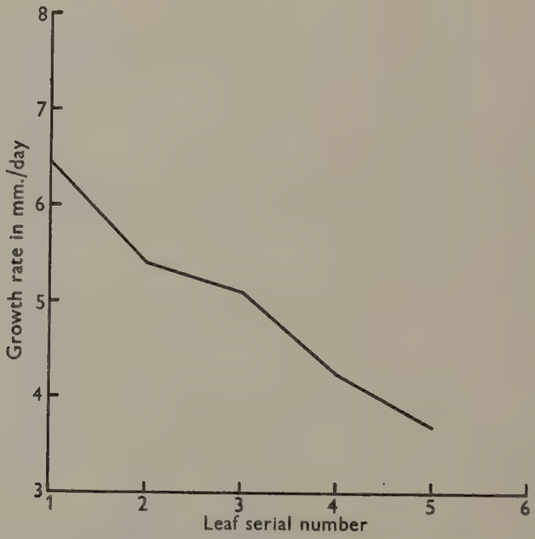

Fig. 4. Growth rates of *P. infestans* hyphae in leaves on potato plants shortly before flowering.

Series of control plants were prepared by inoculating the leaflets on the lowest five leaves, without excision, and subsequently treating them with the experimental series. The experiments were carried out on plants at various developmental stages, but not simultaneously on plants at different stages.

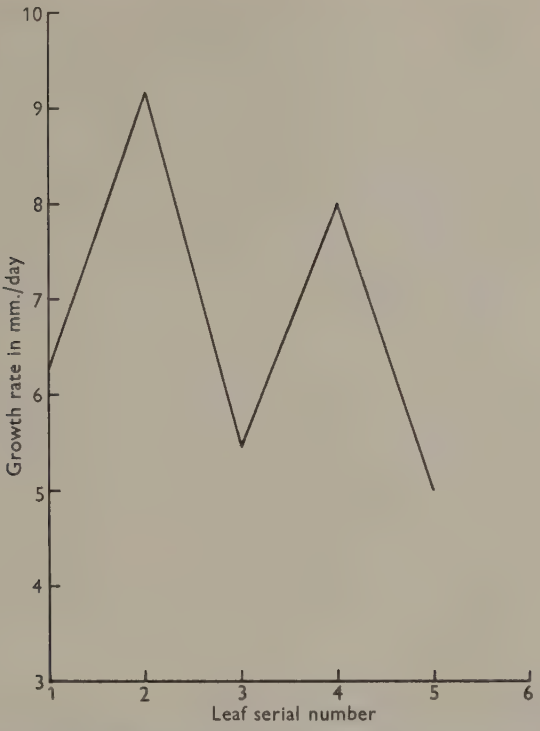

Fig. 5. Growth rates of *P. infestans* hyphae in leaves of potato plants shortly before flowering; leaves 2 and 4 excised and maintained in distilled water.

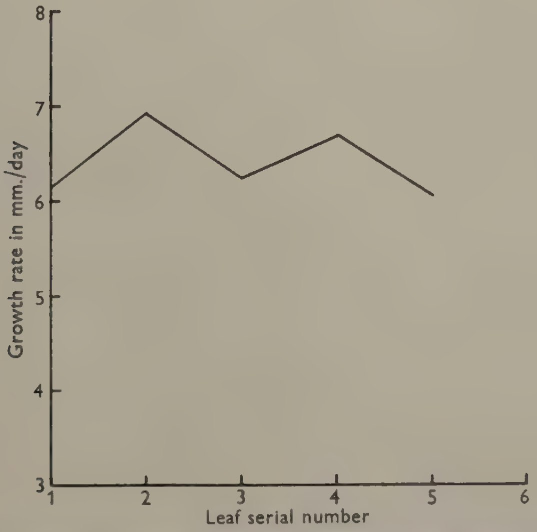

Fig. 6. Growth rates of *P. infestans* hyphae in leaves of potato plants after flowering; leaves 2 and 4 excised and maintained in distilled water.

Experimental results

Mean hyphal growth rates for each leaf in a control series, and in series of experimental plants prepared and inoculated before and after flowering, are illustrated in Figs. 4–6 respectively. It was shown, by analysing the regressions, that the relationship between the rate of hyphal growth and the position of the leaf on the plant was negatively linear (linear F highly significant) in the controls; therefore the occurrence of a significant quartic effect following the excision of the second and fourth leaves would indicate that the hyphal growth rate in the excised leaves had been affected by this treatment. The hyphal growth rate increased by 3·07 mm./day (quartic F very highly significant) in the leaves excised from the younger plants, and by 0·575 mm./day (quartic F significant) in leaves excised from the older, post-flowering, plants, as compared with growth of the hyphae in the leaves remaining on the plants in each series. The reduced effect on hyphal growth of excising leaves in the older plants may be due to excision not accelerating the process of senescence to any great extent, once it had started naturally. Thus the curve in Fig. 6 is almost horizontal, indicating that the conditions previously found in the oldest series of plants illustrated in Fig. 3 were by then developing, whereas the more steeply descending curves in Figs. 4 and 5 are typical of younger plants.

Discussion

The results presented in this paper indicate the occurrence of three interrelated factors affecting the susceptibility of Majestic potato leaves to *P. infestans*, namely nitrogen nutrition, host senescence and hyphal growth rate in the tissue. Schaffnit & Volk (1927) have recorded that, in their experiments, high nitrogen nutrition increased the resistance of potato plants to blight while host senescence decreased it. Vowinckel (1926) also found increased susceptibility associated with maturity in his potato plants. If host senescence is of importance, it is reasonable to suppose that nitrogen nutrition of the host should also show effects, so long as additional dressings of nitrogen promote vegetative growth and delay senescence. It is interesting that changes associated with the early stages of senescence can cause accelerated growth of the parasitic hyphae during both naturally and artificially induced ageing of leaves, as this supports the results of the preliminary observations on the original field plots, in suggesting the presence of a chemical mechanism either inhibiting or stimulating hyphal growth at different stages of host development. This type of mechanism could well be connected with the nutrition of the parasite and independent of any toxins produced in necrotic cells, particularly since *P. infestans* is known to be highly specific in its nutrient requirements. A condition of 'hypersensitivity' could occur, due either to extremely rapid production of toxins in the dying host cells, or to unsuitable nutrition so slowing the rate of hyphal development that the hyphae could not escape from an area even of slow toxin production.

Majestic potatoes are normally moderately 'field resistant' to potato blight, and their resistance may, in consequence, be more readily altered than that of either very resistant or very susceptible varieties. However, these results agree broadly with the observations made by Grainger (1956) that the potato plant passes through phases in its resistance to blight. In the present experiments young plants were shown to have a low average resistance, while more mature but not yet flowering plants were especially susceptible only in the lower leaves. These lower leaves may be attacked relatively early in the field whereas the main blight epidemic may not develop until the upper leaves become susceptible; this change appears to correspond approximately with the flowering period, although the exact correspondence with plant development has not yet been fully investigated. It would, nevertheless, correspond quite closely with the mid-June period at which the Beaumont blight forecasting system becomes effective.

We wish to thank Dr R. C. Campbell for the statistical analysis of the field experiments, Mr W. J. Ridgman for his assistance in statistical analysis of the glasshouse experiments, Miss Kostrovicka for providing cultures of *P. infestans* and the Statistical Department of the Rothamsted Experimental Station for carrying out some of the calculations on the N.R.D.C. 401.

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THE OCCURRENCE OF *PEZICULA ALBA* SP.NOV. AND *P. MALICORTICIS*, THE PERFECT STATES OF *GLOEOSPORIUM ALBUM* AND *G. PERENNANS, IN ENGLAND*

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Pezicula malicorticis (Jackson) Nannfeldt, the perfect state of *Gloeosporium perennans* Zeller & Childs, previously found only in the United States and Holland, is reported on apple trees at Horsmonden, Kent. The perfect state of *G. album*, previously unknown, is described and given the name *Pezicula alba* sp.nov. Both fungi were grown from ascospores and the pathogenicity of these cultures to apple was demonstrated.

INTRODUCTION

Apothecia of *Gloeosporium perennans* were first observed by J. S. Cooley in the United States in 1928 (unpublished) and later described by Kienholz (1939), who considered that the fungus was very similar to *Neofabraea malicorticis* Jackson, the casual organism of 'anthracnose', but he retained the specific difference and gave it the name *N. perennans*. Apothecia associated with *G. perennans* have recently been reported from the Netherlands (Bolay, 1956) under the name *Pezicula malicorticis*, but have not previously been reported in England. No apothecial state has yet been reported for *G. album*.

During the summers of 1955 and 1956 a search for the perfect states of *G. perennans* and *G. album* was carried out as part of a general investigation into the distribution and spread of the two species. No apothecia were discovered during 1955, but it was realized that very large numbers of dead pruning snags and fruiting spurs were infested by one or other of these two fungi (Guthrie, 1957). This material was thought to be a likely source of the perfect state, as Kienholz had reported the development of apothecia on old acervuli. Material which was producing large numbers of conidia of *G. album* and *G. perennans* was therefore collected during the summer of 1956 from orchards in the Weald of Kent. Attention was concentrated on the variety Cox's Orange Pippin because it is extensively grown and stored and suffers heavy losses from these fungi; the material collected included pruning snags, fruiting spurs, mummified fruit and, for *G. perennans*, large stem cankers. After examination to confirm the presence of acervuli the material was stored over sand, exposed to the weather except for a cover of muslin, and examined at intervals for the presence of apothecia. In September the material was moved to South Kensington and thereafter stored on the roof of the Botany Department of Imperial College.

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* Now at Plant Breeding Station, P.O. Njoro, Kenya.

For some months these examinations disclosed only acervuli, but apothecia of both species were finally found on 21 November 1956, those of *G. perennans* on pruning snags and large cankers, and of *G. album* on pruning snags only. Further material was collected on 27 November and apothecia found on this also, though in very small quantities, and of *G. album* only. Apothecia of *G. perennans* were found on this material on 12 December, in one instance on a pruning snag which also bore apothecia of *G. album*.

GLOEOSPORIUM ALBUM

Apothecia

It is difficult to give a precise description of the apothecial material, mainly because so little was available for study. As it has been impossible to continue the work in subsequent seasons, it was thought that publication of the information so far obtained would be useful.

Apothecia were found on dead pruning snags taken from trees of both Cox's Orange Pippin and Worcester Pearmain, on areas which had earlier produced large numbers of conidia. To the unpractised eye the ascocarps were almost indistinguishable from acervuli, being approximately 1 mm. diam., sessile, slightly convex, and pale grey or buff. They became swollen when moist and were then more easily visible. Apothecia are apparently often produced in the remains of acervuli, and mixtures of asci and conidia were often found in a single stroma. There was some evidence that apothecia may develop at the base of acervuli and grow up through them; this condition is similar to that reported by Kienholz for *Neofabraea perennans*. It was not possible to cut sections of the limited material available, particularly in view of the difficulty of identifying an intact apothecium.

The asci were clavate and inoperculate, and were interspersed with irregularly branched paraphyses. Ascospores are originally aseptate but become 3- to 5- and occasionally 6-septate. It appeared that the development of septa is a normal part of the maturation process; this contrasts with *N. perennans* for which Kienholz (1939) established that septate spores were the 'products of age or unfavourable environment and are not to be considered typical of the species'. The ascospores were normally constricted at the septa.

Cultural studies

The fungus was studied in pure cultures which were derived from single spores as follows. An apothecium was placed in a drop of water on a microscope slide and observed under a microscope until several asci had discharged their contents. The apothecium was then removed and loopfuls of the ascospore suspension spread on to the surface of a thin layer of filtered plain water agar in a Petri dish, along lines previously marked on the bottom of the dish with a grease pencil. The dish was then incubated at 20° C. until the ascospores were beginning to germinate, when isolated spores were selected under the microscope and transferred to plates of acidified potato dextrose agar using a dummy objective. It was necessary to use an acid medium because of the presence of bacteria; the pH was therefore adjusted to approximately 4·5 with lactic acid. The fungus was able to grow away from bacteria on this medium.

Single hyphal tips were transferred to plates of normal potato-dextrose agar, and at the same time mycelial transfers were made from a stock culture derived from conidia. Subsequent growth was very similar in appearance and growth rate. Cultures derived from single ascospores eventually gave rise to typical conidia of *G. album*.

Inoculations into apple fruits

Cox's Orange Pippin apples were inoculated with mycelium of *G. album* derived from both ascospores and conidia on 2 February 1957 and stored at 10° C. Two methods of inoculation were used.

(1) *The plug method.* A plug of tissue 3–4 mm. by 4 mm. diam. was removed from the apple with a cork borer, a small piece of mycelium from a plate culture placed in the hole and the plug replaced and lightly smeared with 'Vaseline'.

(2) *The flap method.* A flap of skin approximately 1 cm. square was lifted with a scalpel and a small hole dug in the flesh below. Agar plus mycelium from a plate culture was then placed in the hole and the flap of skin pressed down and smeared lightly with 'Vaseline'.

Typical *Gloeosporium* lesions developed on all the inoculated fruits, production of acervuli began after 4 weeks and was general after 5 weeks from the time of inoculation. The ascospore isolates gave rise to typical *G. album* conidia.

There seems no doubt that the apothecia described are those of *G. album.* No apothecial state has been described previously, consequently it is necessary to rename the fungus on the basis of this perfect state. In a revision of the genus *Gloeosporium*, von Arx (1957) refers to the perfect state of *G. perennans* as *Pezicula malicorticis* (Jackson) Nannf. Because of the close similarity between the two species, the perfect state of *G. album* is apparently best referred to *Pezicula* also, and is therefore described as follows:

Pezicula alba sp.nov.

Gloeosporium album Osterwalder, *Zbl. Bakt.* (Abt. 2), **18**, 825.

Apothecia gregaria, sessilia, grisea, disco convexo, marginato, 1 mm. diam. *Asci* clavati, octospori, 125–150 × 13–24 μ, obturaculo apicali jodo coerulescente; *ascosporae* oblongatae vel fusoideo-oblongatae, rectae vel levissime curvulae, distichae, hyalinae, 20–30 × 7–10 μ, 3–5(6)-septatae. Paraphyses numerosae, ramosae, sursum leviter incrassatae, crassit, 3 μ. Ad ramulos *Pyri malae*, mense Novembri optime evoluta lecta.

Differs from *Pezicula malicorticis* (Jackson) Nannf. in the slightly larger asci and ascospores, in the ascospores being normally septate at maturity, and the conidia markedly curved.

Apothecia sessile, approx. 1 mm. diam., grey, often developing in the remains of acervuli. *Asci* clavate, inoperculate, 125–150 μ by 13–24 μ (mean 135 by 16·2 μ); *ascospores* 20–30 μ by 7–10 μ (mean 24·4 by 8·4 μ), at first aseptate, becoming 3- to 5- and occasionally 6-septate, biseriate. Developing in the autumn and winter on dead tissues of *Pyrus malus* L. following the conidial state, *G. album*.

Type locality: Horsmonden, Kent, England.

The type specimen, consisting of microscope slides only, has been deposited in the herbarium of the Royal Botanic Gardens, Kew, Surrey.

GLOEOSPORIUM PERENNANS

Apothecia

Material bearing apothecia of *G. perennans* was limited, but there is little doubt that the apothecia found were identical with the description given by Kienholz (1939) for *Neofabraea perennans*.

Single ascospore isolations were made by the methods described for *G. album* and gave rise to cultures typical of those produced from conidial isolates. On inoculation into apple fruits the ascospore cultures gave lesions and conidia typical of *G. perennans*.

DISCUSSION

On the present evidence it is difficult to assess the importance of the apothecia of these two fungi in England. Very little apothecial material was found in the 1956–57 season, but a more extensive search might have led to the discovery of apothecia in larger numbers. It has, however, been established (Guthrie, 1957) that apple trees, in particular those of the variety Cox's Orange Pippin, bear very numerous acervuli living saprophytically on dead pruning snags and fruiting spurs. There is evidence that conidia from these sources are able to spread very efficiently by the combined action of rainsplash and wind (Gregory, Guthrie & Bunce, 1959). It is thus unnecessary to postulate extensive spread of the two species by ascospores in order to account for the high level of fruit infection commonly experienced. Further, apothecia are not essential for the survival of the fungi during the winter. Monthly sampling for the presence in the orchard of active acervuli showed that conidia were produced in large numbers throughout the year with a peak around November.

It may be, therefore, that the perfect states are of academic importance only, but the present evidence is too limited to give any reliable indication on this point.

The work described in this paper was carried out in part fulfilment of the requirements for the degree of Doctor of Philosophy of the University of London. I am indebted to the Department of Scientific and Industrial Research for financial support during the period of the work. I would also like to thank Dr P. H. Gregory for his advice and guidance and Dr R. W. G. Dennis for help on taxonomic aspects, also many other people who have helped in various ways. Finally, my thanks are due to the Hon. Director of Agriculture, Kenya, for permission to publish this paper.

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OBSERVATIONS ON THE PERFECT STATE OF *GLOEOSPORIUM PERENNANS* IN ENGLAND

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(With Plate 30 and 2 Text-figures)

Details are given of the morphology of apothecia produced on apple shoot lesions infected by *Gloeosporium perennans*. The characters of cultures obtained from discharged ascospores and pathogenicity tests have confirmed that the apothecia are those of the perfect state of *G. perennans*. These observations are discussed with reference to the systematic position of the fungus and to the possible importance of apothecia in the etiology of the disease.

INTRODUCTION

Gloeosporium perennans Zeller & Childs was first recorded in this country by Wilkinson (1945) as the fungus causing canker and dieback lesions on apple shoots and as one of the three species of *Gloeosporium* responsible for bitter rot of stored apples. Although conidia of this organism are readily produced by both wood and fruit infections in this country, it was not until 1957 that Guthrie recorded the perfect state of *G. perennans* on naturally occurring infected wood which had been collected several months earlier from orchards in south-east England. The present studies, made during recent work on the life-histories of the bitter rot fungi (Sharples, 1958), give details of the structure of the fructifications and of the cultural characters and pathogenicity of ascospore isolates obtained from ascigerous material recorded at Long Ashton during 1957.

MORPHOLOGY

At the beginning of April 1957 branches with natural infections arising from pruning wounds were collected from Allington Pippin bush apple trees and stored at room temperature in a moist chamber. After 2 weeks, three of the lesions, from 1 to 3 cm. long, produced apothecia which were morphologically similar to those described by Kienholz (1939).

In October 1957 large numbers of apothecia were produced on eighteen cut branches by infections, artificially induced by pure culture inoculations with mycelium or conidial suspensions of *G. perennans* in the autumn of 1955 and 1956 on both Cox's Orange and Allington (Pl. 1). The branches had been detached in December 1956 and stored out-of-doors for 9 months on grass or sand in wire cages. Abundant ascigerous material was produced again in July 1958 on artificial branch infections of November 1957 detached in May 1958.

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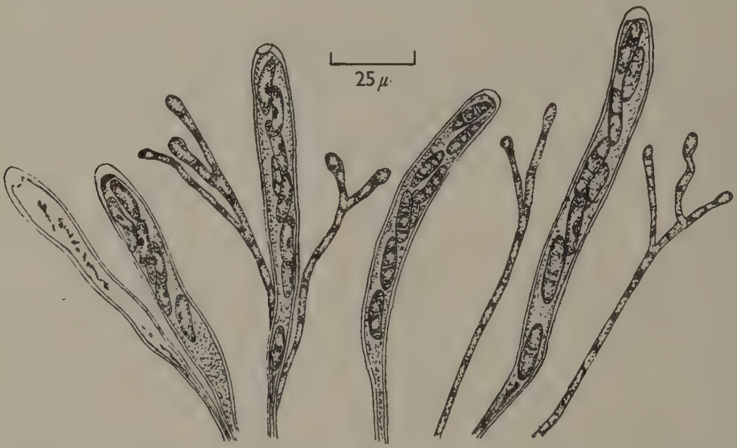
The immature fruiting bodies first appear as raised blisters in the periderm overlying the infected cortex. Alternatively, fructifications may be borne directly on the exposed surface of the infected phloem, where the outer bark has peeled away. The apothecia emerge as small, brown, erumpent structures surrounded by the edges of the ruptured periderm. As the hymenium develops the apothecia become convex and at maturity measure from 0·5 to 1·1 mm. diam. and from 0·25 to 0·4 mm. in height. They are sessile and normally separate from one another; occasionally two or three fructifications may coalesce. They have a waxy appearance and are mostly dark brown, becoming paler under very moist conditions.



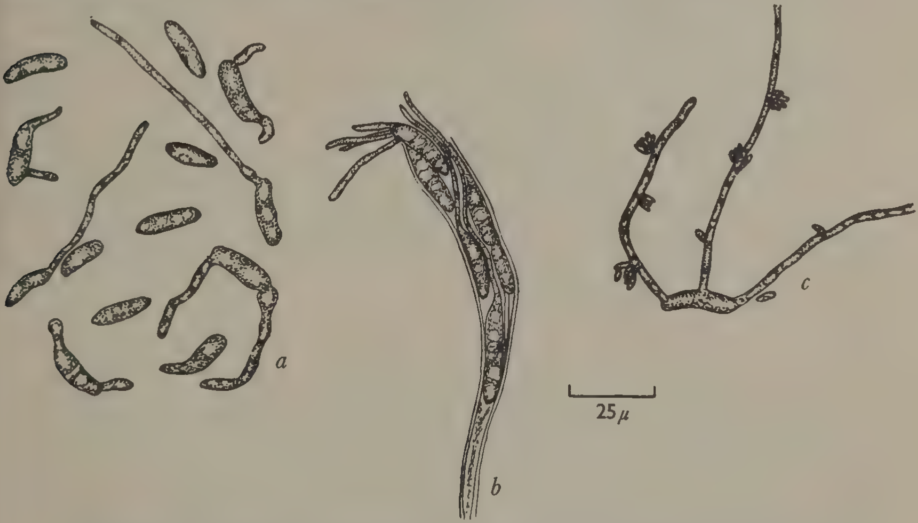
Text-fig. 1. Asci and paraphyses of the perfect state of *Gloeosporium perennans*.

Typical conidia of *G. perennans* were found in the base of old stromata adjacent to apothecia.

The hypothecium consists of loosely interwoven hyphae arising from the intercellular mycelium in the surrounding host tissue. The excipulum is represented by a thin sheath of paraphyses and is lined on its outer surface by the peridermal cells of the host (Pl. 2). The hymenium consists of numerous clavate asci each containing eight ascospores: the asci, which stand parallel, are interspersed by septate, simple or branched paraphyses which are distinctly swollen at their apices. The inoperculate asci measure 86–168 × 8–14 μ, average 121 × 12·5 μ. Usually the ascospore arrangement is roughly biseriate or irregular but is occasionally uniseriate. The hyaline ascospores are normally unicellular but asci containing 1-, 2- and 3-septate spores also occur. The stems of the paraphyses are from 2 to 2·5 μ diam. whilst their apices range from 2·5 to 5 μ diam. (Text-fig. 1). The ascospores are ellipsoidal, often with one side flattened. The contents are usually granular, but there may be a number of large vacuoles or oil droplets. The ascospores measure 13–22·5 × 4·5–8 μ, average 19·5 × 6·7 μ (Text-figs. 1, 2*a*).

The asci mature progressively and the ascospores are forcibly ejected through an apical foramen; their dispersal is by air currents, in contrast to that of the conidia, which are water-borne. Spore discharge continues for several days in moist chambers at room temperature until the apothecia become increasingly gelatinous and eventually deliquesce.

Ascospores produce germ tubes within 24 hr. when discharged into moisture or on to a thin film of plain agar on coverslips. Occasionally several may germinate within the ascus (Text-fig. 2*b*). The germ tubes are normally produced from one or both ends of the spore and sometimes a third may arise from the spore wall near a septum (Text-fig. 2*a*). The germ tubes become septate and grow into normal hyphae. During the following 24 hr., growth is rapid and branches are frequently produced. At the same time a few secondary conidia may be budded off from the walls of the hyphae. During the next few days, large numbers of secondary conidia are produced in clusters along the lengths of the hyphae: their production is particularly abundant on 2 % malt-extract agar (Text-fig. 2*c*). These secondary conidia are within the size range given by Wilkinson (1945) for secondary conidia produced in cultures of *G. perennans*. When washed off into a watch-glass containing distilled water, about 50 % normally germinated.



Text-fig. 2. Germination of ascospores: (*a*) 24 hr. after discharge on to plain agar; (*b*) within the ascus; (*c*) production of secondary conidia from germ tubes.

Over fifty cultures were established from isolated ascospores. These closely resembled cultures established from conidia of *G. perennans* in colour, rate of growth and in their production of secondary conidia.

PATHOGENICITY

(*a*) *Fruit.* On 17 May 1957 three Golden Delicious apples were each inoculated in three wounds with mycelium obtained from single-ascospore cultures. Similar numbers of fruit from the same sample were either inoculated with mycelium from a conidial isolate or wounded in three places and left uninoculated. After 4 days at 10° C. the fruit was placed in a store at 5° C. Within 3 weeks the inoculated fruits were showing typical bitter rot lesions and after 5 weeks acervuli of *G. perennans* were produced on the surface of the rots. No lesions developed on the uninoculated fruit.

Inoculations carried out in September 1957 with Worcester and Cox's Orange apples confirmed the relationship between the ascigerous and conidial states.

(*b*) *Branches.* On 7 June 1957 nine freshly made, comparable cuts on Cox branches were inoculated with mycelium of three ascospore cultures and protected by Stericrepe bandage for 3 weeks. A further five inoculations were made with a stock culture of *G. perennans* and five wounds were left uninoculated. By the end of July acervuli of *G. perennans* were being produced in abundance on infections arising from the ascospore cultures. No infections developed from the uninoculated wounds.

A further series of branch infections was initiated in mid-November 1957, by inoculating wounds on twenty-four Cox shoots with mycelium or conidia obtained from a range of ascospore isolates. These lesions developed into extensive cankers of *G. perennans* which, 2 months after detachment from the trees at the end of May 1958, produced large numbers of apothecia identical with those described above.

Discussion

In 1925 the American workers, Zeller and Childs, listed the characteristics of *G. perennans* and gave details of the wood infection phase, which they called 'Perennial canker'. Kienholz in 1935 inoculated apple branches with *G. perennans* and recorded apothecia on the resulting lesions, for which he suggested the name *Neofabraea perennans* (Zeller & Childs) Kienholz (Kienholz, 1939).

In October 1956 Bolay, working at Wageningen, Netherlands, recorded a perfect state associated with both natural and artificial infections of *G. perennans* on apple trees in several localities in the Netherlands. Towards the end of the following month, Guthrie (1957) also recorded apothecia on naturally occurring cankers and snag infections of *G. perennans* which had been collected in the previous summer from orchards in south-east England.

The results of the pathogenicity tests and cultural characters of ascospore isolates confirm that the apothecial fructifications discovered at Long Ashton belong to *G. perennans*. In Table 1 the published measurements of the perfect state of *N. perennans* as given by Kienholz are compared with those of the Long Ashton material; although the ascospore sizes of the latter are somewhat larger than those given by Kienholz, this difference is not considered sufficient to distinguish the Long Ashton material from *N. perennans*.

Full details of the perfect state of *G. perennans* in the Netherlands have not been published by Bolay. However, from examination of a small sample of ascigerous material found at Druten and sent to Long Ashton it was established that Bolay had been dealing with the same fungus.

There has been considerable discussion on the distinction between

N. perennans and a related species *N. malicorticis* (Cordley) Jackson, which causes anthracnose rot in apples in addition to an apple canker disease. Kienholz states that the apothecia of these two species are morphologically indistinguishable, but claims that the perennial canker and the anthracnose diseases show differences in pathogenicity and geographical distribution. It is stated that conidia of *N. malicorticis* are usually allantoid to U-shaped, ranging from 15–35 to 3–6 μ, while those of *G. perennans* are smaller (12–25 × 3–6 μ), mostly cylindrical, rarely curved and usually larger at one end.

Table 1

	Kienholz description	Long Ashton material
Apothecia (mm.)	0·5–1·0	0·5–1·1
Asci		
Range (μ)	50–142 × 8·5–19·8	86–168 × 8–14
Average (μ)	98·1 × 12·6	121 × 12·5
Ascospores		
Range (μ)	11·5–22·7 × 4·2–8·5	13–22·5 × 4·5–8
Average (μ)	17·7 × 6	19·5 × 6·7
Paraphyses		
Diam. at apex (μ)	< 7	< 5

Since the recent discoveries of the perfect state of *G. perennans* in Europe, discussion has restarted concerning its correct name. A number of workers disagree with the distinction between *N. malicorticis* and *N. perennans* made by Kienholz (1939) and regard the two as synonymous, particularly since the shoot infection stage is not truly perennial (McLarty, 1933). The generic name preferred in the U.S.A. is also disputed by European workers, since Nannfeldt (1932) incorporated *Neofabraea* Jacks. in *Pezicula* Tul. Although later work in America (Thompson, 1939) indicated that these two genera were distinct, Bolay (1956) prefers *P. malicorticis* (Jackson) Nannfeldt and states that this is the same as *N. malicorticis* (Cordley) Jackson.

Since adequate material of the disease causing apple anthracnose in America is so far unavailable in this country, no comparative work on the systematics of these fungi has been undertaken here.

Kienholz has shown that apothecia are not usually produced until the lesions are in their second year at least and he pointed out that they were most abundant on uprooted dead trees. All the records of the perfect state of *G. perennans* in this country have so far been confined to detached infected wood (Guthrie, 1957 and above) and it seems probable that the imminence of complete exhaustion of nutrients in the host tissue stimulates the production of apothecia.

Spread of the disease within an orchard is normally effected by conidia which are dispersed in rain splashes. More widespread dispersal of the disease, possibly for several miles, to previously uninfected orchards may take place, however, when airborne ascospores are discharged from apothecia developing on old prunings. Although the relative importance of dissemination of the disease by ascospores has yet to be studied, it is clearly advisable to burn all excised wood infected by *G. perennans* with minimum delay.

Grateful thanks are due to Mr R. W. Marsh and Dr R. W. G. Dennis for their interest and advice throughout this work, to Prof. A. J. P. Oort of the Phytopathology Laboratory, Wageningen, for kindly supplying a sample of the Dutch ascigerous material and to Miss N. M. Waugh for technical assistance. I am also indebted to Mr D. Aliband and Mr J. Collyer for the photographs.

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EXPLANATION OF PLATE 30

Fig. 1. Mature apothecia on detached shoot infected by *Gloeosporium perennans*.

Fig. 2. Vertical section through apothecium of perfect state of *G. perennans*.

(*Accepted for publication* 9 *February* 1959)

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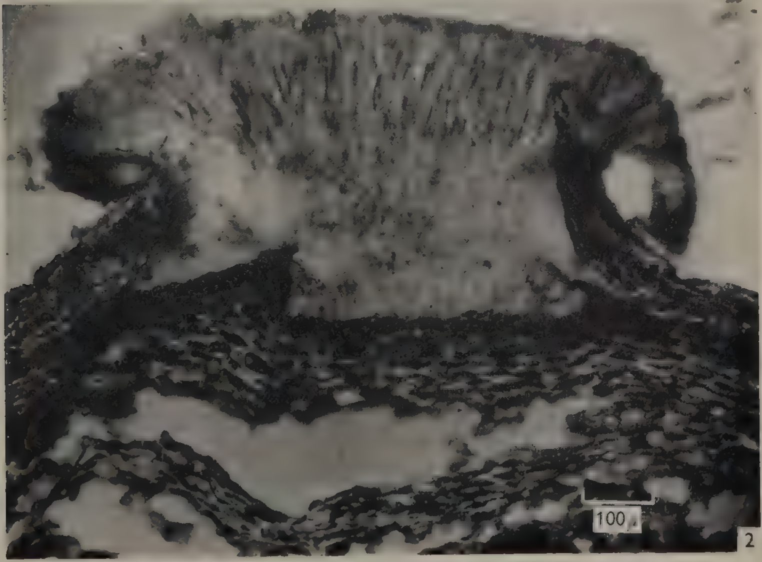

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Trans. Brit. mycol. Soc. **42** (4), 513–524 (1959).

A NEW VARIETY OF *PHYTOPHTHORA ERYTHROSEPTICA*, WHICH CAUSES A SOFT ROT OF PEA ROOTS

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(With 6 Text-figures)

The morphology and pathogenicity of a new variety of *Phytophthora erythroseptica* is described. The fungus was isolated from pea roots in the glasshouse although it was derived, almost certainly, from field soil. Infected roots were soft, sodden and faintly discoloured; pea shoots wilted when root infection was severe. Peas were infected with the fungus, either by planting seed in soil inoculated with agar disks containing growing mycelium, or by immersing washed root systems in a suspension of zoospores. The zoospores encysted on the root tips, where they germinated and penetrated the host. Inter- and intracellular hyphae grew first along the stele and then into the cortex. Abundant sex organs usually formed in infected tissue and sporangia grew out from the roots. Infection was mild out-of-doors in the summer and was very severe in the glasshouse at 15° C.

Introduction

A species of *Phytophthora* was found in the roots of peas growing in a mixture of field soil and compost in the glasshouse at 15° C. and, after some difficulty, was isolated on bean agar. In possessing amphigynous antheridia and non-papillate sporangia the isolate resembled most closely *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. himalayensis* and *P. richardiae*; direct comparisons of morphological and physiological characters were made with all the isolates of these species which were obtainable. The pea isolate was clearly distinct from all these authentic isolates and also from another similar species, *P. vignae*, described by Purss (1957) after this work was carried out, and so it is described here as a new variety of *P. erythroseptica* and named *P. erythroseptica* var. *pisi*.

Pathogenicity experiments

Pathogenicity to peas

Proof of the pathogenicity of the pea isolate to peas was obtained from several infection experiments. Early Bird peas were grown at 15° C. for 11 days in sterile compost in 5 in. pots. By this time they were 3–4 in. in height and five pots were placed at each of four different temperatures, respectively, as follows: 15 and 10° C., in glasshouses; in a cold frame; and outside with temperatures ranging from 2 to 20° C., averaging 10° C. After 2 days, three pots from each batch of five were inoculated by placing twelve 1 cm. disks from a bean agar culture just under the surface of the compost. The remaining two control pots were treated similarly with sterile agar. All pots were watered regularly up to the brim.

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Fourteen days after inoculation there were no differences in appearance of the aerial parts of the inoculated and control plants, but root tips of the inoculated plants at 15° C. were flaccid and discoloured a pale yellowish grey. The roots of four plants from each series were stained by boiling for 2 min. in 0·1 % acid fuchsin in lactophenol, cleared in lactophenol overnight and then searched for the *Phytophthora*. Sex organs were present along some lateral roots of the plants grown at 15° C. and at 10° C. No sex organs were present in the roots of plants from the cold frame or from outside.

Twenty-five days after inoculation the inoculated plants at 15° C. were about two-thirds the size of the controls and the roots had almost completely rotted away. Sex organs were abundant in both stele and cortex. There were no differences between the inoculated and control plants in the cold frame and outside and there was no evidence of the *Phytophthora* in their roots.

Thirty-six days after inoculation the inoculated plants at 15° C. were wilting and turning yellow whereas the control plants were healthy. At 10° C. the inoculated plants resembled those at 15° C. 25 days after inoculation. The plants in the cold frame and outside remained unaffected by the fungus. That this was an effect of temperature alone was confirmed in a further experiment in the warmer weather of July, when plants in a cold frame and outside became mildly infected.

In another inoculation experiment at 15° C. in which the compost was inoculated with agar disks at the time when Early Bird peas were planted, the disease symptoms appeared earlier and after 13 days sex organs were present in the radicles along almost their entire length. Similar results were obtained using unsterile compost and with another pea, var. Laxton's Superb.

These experiments demonstrated that infection was severe at 15° C., comparatively mild outside at summer temperatures and absent outside in the temperatures of March and April.

Pathogenicity to other legumes

During the spring and early summer of 1958 two experiments were made to test the pathogenicity of *P. erythroseptica* v. *pisi* towards single varieties of other legumes commonly grown in this country. These were broad bean, var. Longpod Conqueror, dwarf bean, Canadian Wonder, field bean, Stourbridge, runner bean, Prizewinner and sweet pea, Gold Prize. In all essentials of technique these experiments were replicas of the first tests of pathogenicity towards peas. Laxton's Superb peas, included in both tests for comparison, showed typical symptoms of the disease approximately a fortnight after inoculation but, with the exception of the broad beans, none of the other plants showed any evidence of infection whatsoever. Some, but not all, of the broad bean plants showed slight chlorosis of the lower leaves. A proportion of the roots of all the plants bore black lesions extending for varying distances backwards from the ips. Sex organs, characteristic of the fungus, were found commonly in hese roots and, in sterile distilled water, sporangia grew out from the oots in 1–2 days at laboratory temperature. The roots of control plants emained healthy. Although attempts to re-isolate from infected roots were nsuccessful, there can be no doubt that the fungus is pathogenic to broad ean. Under the conditions of the experiment, however, even in plants kept for several weeks, root destruction did not induce any symptoms other than the chlorosis referred to above.

Pathogenicity to other plants

Further pathogenicity tests were made with apple fruits of the varieties Lord Lambourne and White Winter Pearmain, with potato tubers of the varieties Majestic and Arran Victory and with young tomato plants, var. Market King. The apples and potatoes were inoculated with mycelium in agar introduced through wounds while the tomato plants were inoculated: (*a*) by placing mycelium in agar just below the surface of the soil in which the plants were growing and watering frequently; (*b*) by wound inoculation in the foot region. No infection occurred in any of these tests.

PATH OF INFECTION IN PEAS

The path of infection was traced using (1) stained whole root systems, and (2) microtome sections.

Observations on stained root systems

Early Bird peas, 13 days old, were inoculated with a suspension of zoospores prepared in 5 % peat percolate by addition of disks of bean agar permeated with actively growing mycelium. The washed root systems of twelve plants were immersed above the cotyledons in the zoospore suspension and left overnight at 20° C. On the following day five plants were planted in sterile compost and grown at 15° C., four plants were left in the suspension and the remaining three were removed and stained with acid fuchsin.

Staining showed that the encysted zoospores were often very numerous near the root tips, where they sometimes formed a complete sheath up to four layers deep, and were scattered thinly elsewhere on the roots and on the bottom of the Petri dish. Counts of cysts in one plane of focus along one side of each of ten roots were made at 0·25 mm. intervals, starting from the root tip. The averaged results are shown in histogram form in Fig. 1. Penetration of the host root occurred only in the zone of encystment near the root tip. A single germ tube from each cyst grew through the cortex and the contents of the cyst passed gradually into the germ tube, leaving a vacuolated and finally empty cyst. Before germination, the cyst was touching the root, but by the time that the germ tube had penetrated the cortex the cyst had become separated from the root by a short length of germ tube.

The root systems of the four plants which had been left in the percolate suspension were stained and examined 6 days after inoculation.

Numerous sporangia were present behind the root caps in the zone where penetration had occurred. Young sex organs were present in the stele. The five inoculated plants in the compost became infected and the symptoms were as described previously. These observations on the encystment and penetration of zoospores and on the formation of sporangia are very similar to those of Goode (1956) on infection of strawberry roots by *P. fragariae.*

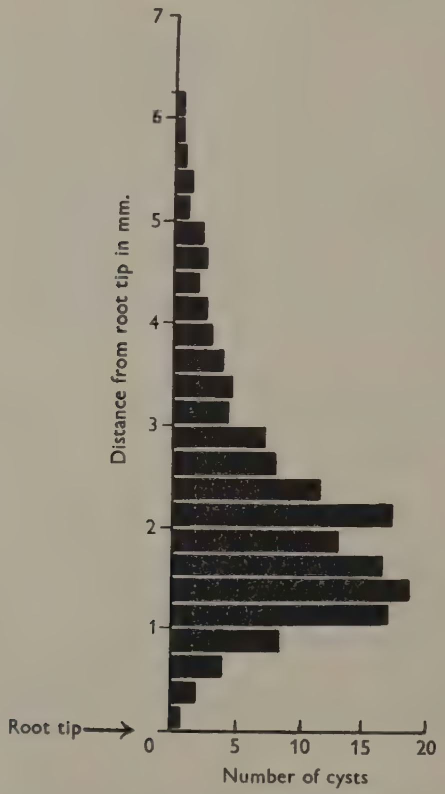

Fig. 1. Frequency distribution of cysts of *P. erythroseptica* var. *pisi* on pea roots.

The progress of infection was studied further by watering pots of Early Bird peas, 11 days old and growing in sand at 15° C., with a suspension of zoospores in 5 % peat percolate. Five days after inoculation the root systems of three plants were stained and examined. The position of the fungus in the roots was assessed approximately by the distribution of sex organs. These were abundant in one-half to two-thirds of the tap roots and lateral roots and were confined to the steles, except at the tips of the roots where they were also present in the cortex. Some root tips had rotted off. Sporangia were present occasionally. Observations made at intervals during the following 16 days revealed that *P. erythroseptica* var. *pisi* had grown along the stele as far as the epicotyl but not beyond. Backwards from the root tip the cortex became progressively invaded from the infected stele. Where such cortical invasion had occurred, the root was flaccid and discoloured pale yellowish grey. Where only the steles were infected, the roots remained turgid and white. There was no pronounced or characteristic discoloration of the root tissue at any stage. Three weeks after inoculation, sex organs were present in the stele and cortex of the whole of each root system.

Observations on microtome sections

Limited observations were made from microtome sections. The root systems of healthy Early Bird peas were immersed in a suspension of zoospores in 5 % peat percolate, as before. Root tips were excised after 5, 48, 68 and 144 hr., respectively, and fixed in formalin–acetic–alcohol.

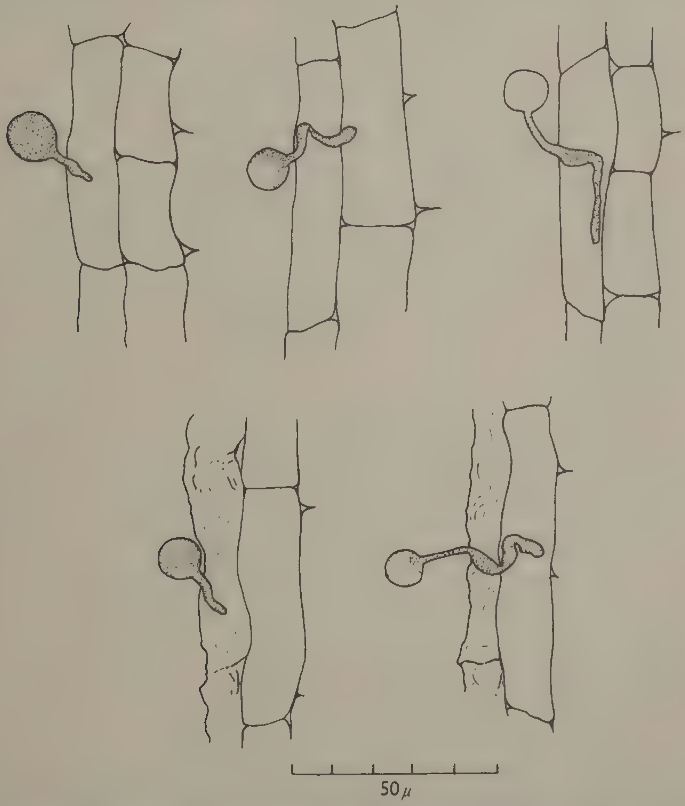

Fig. 2. Germinated cysts of *P. erythroseptica* var. *pisi* penetrating pea root tip. The lower two are penetrating the remains of the root cap layer. Longitudinal section 15 μ thick, stained with acid fuchsin in lactophenol.

After embedding the material in paraffin wax, sections 15 μ thick were cut and stained with acid fuchsin in lactophenol.

After 5 hr. many of the cysts had germinated and penetrated the root. The germ tubes grew through the cell walls, both of the root cap cells, which were sloughing off, and of the epidermal cells farther back, as shown in Fig. 2. After 48 and 68 hr., inter- and intracellular, branching hyphae were present in the stele. The hyphae were often tortuous and of uneven diameter, similar to those in agar, and they were frequently constricted where they passed through a cell wall. After 144 hr., sex organs were present in the cortex and in the tissues of the stele outside the xylem.

MORPHOLOGY AND GROWTH OF THE FUNGUS IN CULTURE

On all the agar media used (bean, clear oat, pea, maize, malt and potato dextrose*) no aerial mycelium was observed, and most of the lateral hyphae were tortuous, and of uneven diameter, ranging from 2 to 14 μ. The remaining hyphae had parallel walls and resembled those of other species of *Phytophthora*. With age they all became sparingly septate.

Oogonia, oospores and antheridia developed 3–4 mm. behind the margins of the colonies on a variety of agar media in the frequencies shown in Table 1.

Table 1. *Production of sex organs of* Phytophthora erythroseptica *var.* pisi *in agar media at different temperatures*

	Temperature in °C.				
	10	15	20	25	30
Bean	o	++++	++++	++++	o
Clear oat	o	++++	++++	++++	o
Pea	o	+	++++	++	o
Maize	o	+	++++	++	o
Malt	o	+	++++	++	o
Potato-dextrose	o	o	+	o	o

o = absent, + = rare, ++ = occasional, ++++ = abundant.

Table 2. *Dimensions in microns of sex organs of* Phytophthora erythroseptica *var.* pisi *in infected pea roots and bean agar, respectively*

(Means of fifty measurements)

	Infected pea roots		Bean agar	
	Mean	Range	Mean	Range
Oogonia	36·5	28–44	35	25–43
Oospores	26·4	14–34	26	20–35
Antheridia (length)	18·8	16–22	19	14–28
Antheridia (breadth)	17·6	11–26	18	14–23

When sufficiently numerous, the sex organs imparted a straw coloration to the colonies. The colour was seen in the walls of the oogonia, oospores and antheridia; it was very pale in the oospore and antheridium walls and more pronounced in the oogonium walls. Oogonia, oospores and antheridia from infected pea root and from bean agar are of similar size, as shown in Table 2.

Stages in the development of antheridia and oogonia are illustrated in Fig. 3. All the antheridia examined were amphigynous and nearly one-third were not separated by septa from the hyphae on which they were borne. The oogonium walls showed a range of thickness, 0·7–2·7 μ, averaging 1·5 μ (thirty measurements) and each oogonium was separated from its hypha by a septum in the stalk. The oospores were spherical and their walls were fairly uniform in thickness, ranging from 1 to 5 μ and averaging 2·9 μ (thirty measurements). At first they were connected to the base of the oogonium by granular, protoplasmic material but, when mature, they lay free inside the oogonium.

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* For the composition and preparation of these and other media see Appendix.

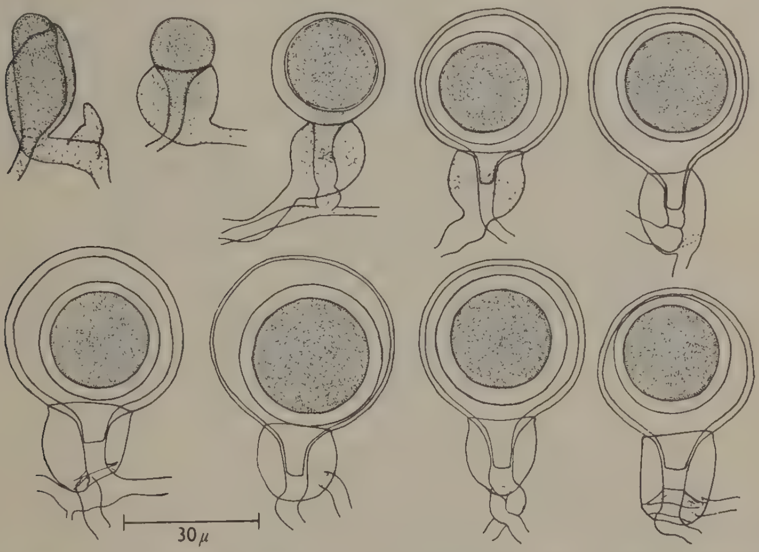

Fig. 3. Antheridia, oogonia and oospores of *P. erythroseptica* var. *pisi* and stages in their development. Drawings made from unstained material in bean agar mounted in water.

Formation of sporangia occurred when colonies of the fungus growing on bean agar were incubated for 2–3 days at ± 18° C. in distilled water, pond water, compost percolate and different concentrations of peat percolate, respectively. Sporangial morphology was alike in all these media but the greatest number of sporangia and zoospores occurred in 5 % peat percolate. In a similar test with Petri's solution (Petri, 1925), the sporangia were not very numerous and many were unhealthy. Sporangia varied considerably in shape from obpyriform to very elongate as shown in Fig. 4. They were non-papillate and non-pedicillate. Germination was normally by zoospores. After appearance of the first formed sporangia, others developed either by sympodial branching of the sporangiophore with the formation of close or lax groups of sporangia, or by extended and/or nested proliferation as shown in Fig. 5. The mean dimensions estimated from measurements of 100 sporangia growing on bean agar in 5 % peat percolate at ± 18° C. were 86 × 35 μ, range 54–171 × 25–40 μ. Active zoospores were biflagellate, reniform and approximately 17 μ in length. Encysted zoospores were spherical, and 11 μ in diameter, range 10–12 μ (fifty measurements).

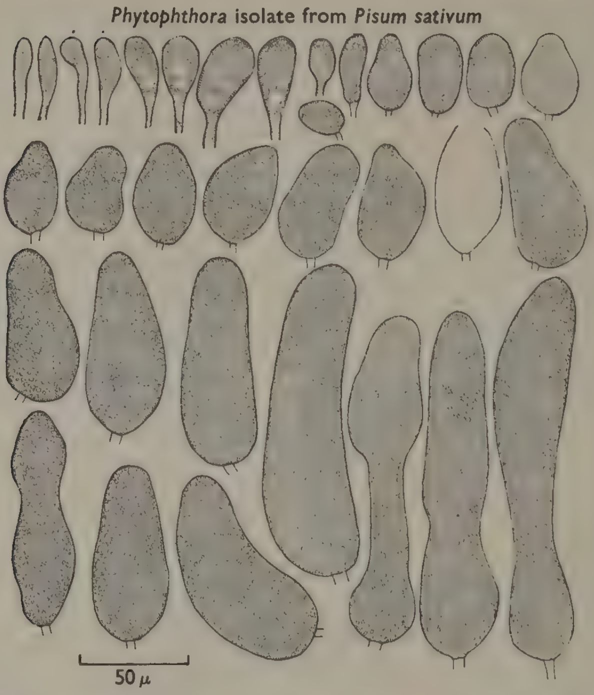

Fig. 4. Sporangia of *P. erythroseptica* var. *pisi* growing from bean agar after 2 days in 5 % peat percolate at ± 18° C.

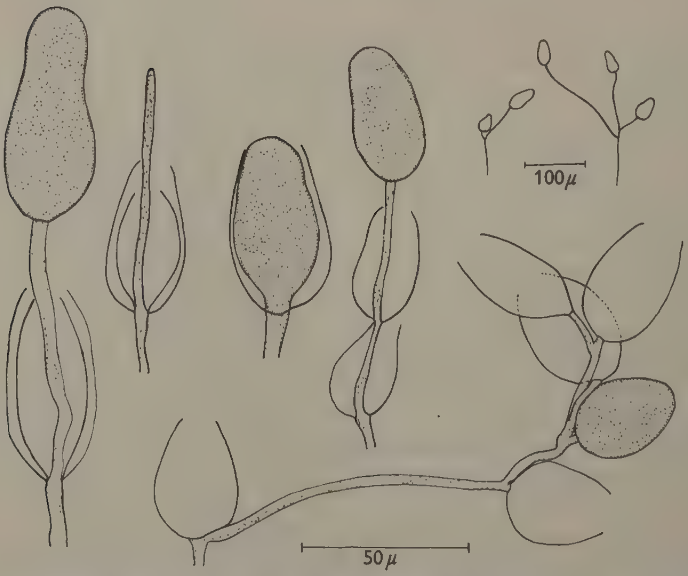

Fig. 5. Proliferation of sporangia and branching of the sporangiophore of *P. erythroseptica* var. *pisi* growing from bean agar in 5 % peat percolate at ± 18° C.

The morphology of sporangia from infected pea roots in tap water, pond water and 5 % peat percolate agreed with that of sporangia formed on bean agar in the various liquid media mentioned above except that they were smaller, 63 × 29 μ, range 43–91 × 19–40 μ (fifty sporangia).

The growth temperature relations of the fungus were investigated on a number of different agar media. Plates of media were each inoculated with a 4 mm. diameter disk cut from the edge of a bean agar culture and incubated in duplicate at a range of temperatures from 5 to 35° C. The media were bean, clear oat, pea and maize-extract agars. Colony diameters, measured after 6 days, are shown in Fig. 6. Growth occurred between 12 and 30° C. and the optimum was 24° C. This range and optimum was the same on each medium though growth was most rapid on bean agar, and slower on clear oat, pea and maize extract agars, in that order. The growth rate on bean agar, however, varied on different occasions.

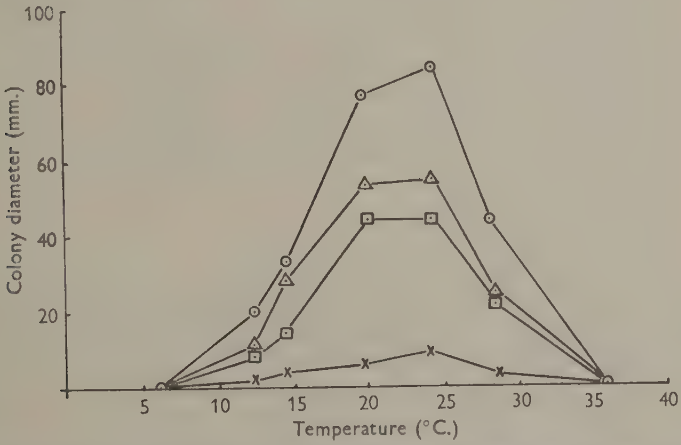

Fig. 6. Growth temperature relations of *P. erythroseptica* var. *pisi* after 6 days incubation in different media. —⊙— French beanmeal agar; —△— clear oat extract agar; —⊡— pea-meal agar; —×— maizemeal agar.

IDENTIFICATION

As stated earlier the pea *Phytophthora* was obviously related to the group of species comprising *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. himalayensis* and *P. richardiae*. It was compared in its morphology, growth in culture and pathogenicity with the authentic isolate of these species listed in Table 3.

While these observations confirmed the close relationship between the *Phytophthora* from pea roots and the named species, the pea isolate was found to differ clearly in a number of characters. These are discussed in detail by Bywater (1956), but may be summarized as follows. The hyphae were extremely uneven in diameter, ranging from 2 to 14 μ, the range in thickness of the oogonium and oospore walls was significantly greater than that of the other isolates and very elongated sporangia occurred in addition to the shorter and more characteristic obpyriform type. The antheridia were large, approximately isodiametric, and about one-third were not separated from their respective hyphae by a septum. Aerial mycelium was lacking from colonies on all the media examined. The temperature range over which the isolate grew, approximately 12–30° C., was considerably less than that of the other isolates. Growth was slow on bean and oat agars and very slow indeed on maize, malt and potato-dextrose agars. Further differences were shown in the pathogenicity tests. Thus the pea isolate was the only one which did not infect Arran Victory potato tubers (except two out of four isolates of *P. drechsleri*) and Lord Lambourne apple fruits, and was the only isolate which attacked Early Bird and Laxton's Superb peas. When compared with the description of *P. vignae* (Purss, 1957), the pea isolate differed morphologically by the possession of very elongated sporangia, by lack of chlamydospores and hyphal swellings, and by the absence of a dark-coloured outer oospore wall. Furthermore, the pea isolate lacked aerial mycelium on all agar media, grew over a narrower range of temperature and had an optimum at 24° C. as compared with one of 28–30° C. for *P. vignae*.

Table 3. *Isolates of* Phytophthora *spp. used for comparison with the new variety of* P. erythroseptica

Isolate	Source
P. cryptogea Pethybridge & Lafferty (1919)	
ex Pethybridge, Tucker	Baarn
ex Tucker	Baarn
ex Tompkins, Middleton	Comm. Myc. Inst.
P. drechsleri Tucker (1931)	
ex Tucker	Baarn
ex Frezzi	Comm. Myc. Inst.
ex Frezzi	Comm. Myc. Inst.
ex Middleton	Comm. Myc. Inst.
P. erythroseptica Pethybridge (1913)	
ex Muskett, Pethybridge	Baarn
ex Hickman	Univ. Birm. Bot. Dep.
ex Lister Institute	Univ. Birm. Bot. Dep.
ex Pethybridge	Comm. Myc. Inst.
P. himalayensis Dastur (1949)	Baarn
P. richardiae Buisman (1927)	Baarn

In a later paper evidence will be presented suggesting that *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. himalayensis* and *P. richardiae* should be grouped together as one species, *P. erythroseptica*, with *P. richardiae* as *P. erythroseptica* var. *richardiae*. It is now proposed to establish the pea isolate as a new variety of *P. erythroseptica* and, since it was first isolated from *Pisum sativum*, it is described below as variety *pisi* of *P. erythroseptica*.

DIAGNOSIS

Main hyphae with parallel walls bearing lateral branches with tortuous growth and uneven diameters, 2–14 μ, averaging 5 μ. Right-angled branching frequently obscured, but constriction at origin of branches usually visible. Aerial mycelium lacking. Oogonia 37 (28–44) μ in diameter with walls 1·5 (0·7–2·7) μ thick. Oospores spherical, 26 (14–34) μ

in diameter with walls 2·9 (1·3–5·3) μ thick. Antheridia amphigynous, 19 (16–22) μ long by 18 (11–26) μ broad, not always separated from their respective hyphae by septa. Sporangia obpyriform to elongate, non-papillate, 63 (43–91) μ long by 29 (19–40) μ broad from pea roots in liquid media, and 86 (54–171) μ long by 35 (25–40) μ broad from bean agar in liquid media. Sporangiophores simple or sympodially branched, new sporangia often arising by proliferation. Germination of sporangia indirect by zoospores. Zoospores biflagellate, reniform, approximately 17 μ long. Encysted zoospores spherical, 11 (10–12) μ in diameter. Parasitic upon *Pisum sativum*, causing a soft rot of the roots.

Phytophthora erythroseptica Pethybridge var. **pisi** var.nov.

Hyphae principales parietibus parallelis multos ramos tortos et diam. irregulares, 2–14 μ, in medio 5 μ, a latere ferentes. Rami rectangulares saepe latent sed in origine ramorum constrictio plerumque visibilio. Aerium mycelium nullum. Oogonia diam. 37 (28–44) μ parietibus 1·5 (0·7–2·7) μ crassis. Oosporae sphericae, diam. 26 (14–34) μ, parietibus 2·9 (1·3–5·3) μ crassis. Antheridia amphigena 19 (16–22) μ longa, 18 (11–26) μ lata, septis a suis quaeque hyphis non semper separata. Sporangia obpyriformia aut elongata, non-papillata, e pisorum radicibus in liquore 63 (43–91) μ longa, 29 (19–40) μ lata, ex fabae agare in liquore 86 (54–171) μ longa, 35 (25–40) μ lata. Sporangiophora simplices vel sympodice ramosa, sporangia nova saepe per proliferationem orientia. Sporangia per zoosporas indirecte germinantia. Zoosporae biflagellatae, reniformes, circa 17 μ longae. Zoosporae encystae sphericae, diam. 11 (10–12) μ. Parasitica in Piso sativo efficiens 'root soft rot'.

DISCUSSION

A number of species of *Phytophthora* has been recorded infecting peas either naturally or after artificial inoculation, but this is the first record of a *Phytophthora* occurring on peas in Britain. *P. parasitica* was isolated from peas in Puerto Rico by Matz (1920) and in Brazil by Müller (1934). *P. palmivora* and *P. faberi* were reported by Reinking (1923) and *P. cambivora* by Arnaudi (1928) to infect peas after artificial inoculation. A *Phytophthora*, which was neither identified not isolated, was reported by Coombes & Julien (1949) to cause a collar rot of peas in Mauritius. *P. vignae*, which caused a stem and root rot of cowpeas (*Vigna sinensis*) in Australia, was isolated and described by Purss (1957).

The species of *Phytophthora* named above are quite distinct from *P. erythroseptica* var. *pisi*. *P. parasitica*, *P. palmivora* and *P. faberi* form papillate sporangia and chlamydospores. The oogonia of *P. cambivora* are considerably larger than those of *P. erythroseptica* var. *pisi*. Petri (1925) reported the diameter as 56–62 μ, and Ashby (1929), who found only three, as 47 μ. *P. vignae*, unlike *P. erythroseptica* var. *pisi*, possessed chlamydospores, hyphal swellings and smaller sporangia and antheridia, and differed in cultural characteristics and growth temperature relations.

P. erythroseptica var. *pisi* was found infecting peas growing in compost containing organic matter from field soil cropped with peas, collected near Worcester. Since infection with this fungus did not occur in compost alone, it seems that it originated from the field soil, although it has not yet been identified in the field.

In the early stages of infection of peas by *P. erythroseptica* var. *pisi*, the root symptoms were identical with those produced by *Aphanomyces euteiches* and by *Pythium debaryanum*, which also were isolated from pea roots in this work. In a small inoculation test in which these three fungi, respectively, infected Early Bird peas growing in unsterile compost at 7° C., infection was mild after 3 weeks and root tissue infected by each fungus was identical in appearance. In very old root tissue infected with *Aphanomyces euteiches*, the oospores often remain the only visible evidence of the fungus and these closely resemble *Phytophthora* oospores in appearance and dimensions. Despite these similarities, the pea isolate can be readily identified by means of its oogonia bearing amphigynous antheridia.

We wish to thank Mr A. D. Fitton-Brown of the University College of North Wales, Bangor, for the Latin diagnosis.

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Appendix

Each agar medium contained 2 g. agar per 100 ml. water and was sterilized by autoclaving at 15 lb. pressure for 15 min.

Clear oat extract agar. Extract 5 g. oatmeal in 50 ml. water for 2 hr. at 60° C. Filter through coarse filter-paper and add agar dissolved in water.

French beanmeal agar. Boil 3 g. beanmeal for 10 min. in 50 ml. water and add agar dissolved in water.

Maizemeal agar. Preparation as for French beanmeal agar.

Malt-extract agar. Dissolve 2·5 g. malt extract in 50 ml. water and add agar dissolved in water.

Peameal agar. Preparation as for French beanmeal agar.

Potato-dextrose agar. Boil 20 g. potato tissue, sieve through muslin and add 2 g. dextrose and agar dissolved in 50 ml. water.

5 % *peat percolate.* Leach roughly estimated volume of peat with an approximately equal volume of distilled water. Dilute the percolate 20 times with distilled water.

(*Accepted for publication* 11 *March* 1959)

REVIEWS

Fungal Diseases of Animals. By G. C. AINSWORTH and P. K. C. AUSTWICK. (Review Series No. 6 of the Commonwealth Bureau of Animal Health. Farnham Royal, Bucks, England: Commonwealth Agricultural Bureaux, 1959.) Pp. xii + 148, 4 coloured plates, 25 figures. Price 20*s*.

The study of medical mycology has made great strides during the past twenty years, and during this time several books have been published on the subject in the English language. The author of one of these, Dr G. C. Ainsworth, is now co-author with Mr P. K. C. Austwick of this, the first comprehensive book on diseases of animals caused by fungi. These authors have been, in recent years, in the forefront of the intensive drive to investigate mycotic disease in animals in Britain, and have been mainly responsible for encouraging others to take up the study of veterinary mycology. Members of the veterinary profession are particularly fortunate that both authors are so well qualified to write about their subject, both being well trained and widely read in the many ramifications of applied mycology. The general plan of the book is reminiscent of that found in the *Manual of Clinical Mycology* by Conant, Smith, Barker, Callaway and Martin (1954), but since it concerns animals the subject-matter is much wider in its scope. Its chapters include all the mycoses of animals known in Britain as well as those which are restricted to the Western Hemisphere, such as North American Blastomycosis, Coccidioidomycosis and Histoplasmosis (North American type). The importance to man of the study of mycoses in animals derives not only from the fact that a number of them can be transmitted by animals to man, but also because, for obvious reasons, they can be more frequently and more readily studied in animals than in man. These authors have been particularly fortunate in this respect, having had, in fact, more material at their disposal than they could conveniently handle. The chapters on Aspergillosis and Moniliasis should have a special appeal, alike to members of both the medical and veterinary professions, not only because they are the most widespread of the systemic mycoses in both man and animals, but also because they have been studied intensively by these authors. The sixteenth chapter is devoted to the toxicoses caused by mouldy food materials and by the larger poisonous fungi, a subject which has received more attention in Russia than in other countries. This is followed by the closing chapter which deals with Ergotism. Students of medical and veterinary mycology can rely on this text as being a model in its approach to the subject of mycotic disease, based as it is on a sound nomenclature and a sound knowledge of the pathogenic fungi in both their saprophytic and their parasitic phases. Research workers will find, in addition to the immense amount of information which is condensed in this little book, a long list of references to the best literature on medical and veterinary mycology. A number of excellent photographs and photomicrographs illustrating clinical and pathological features includes four striking plates in colour each bearing two figures. The text is well printed on good quality paper and is bound in blue cloth. The price is so low that it should be within the reach of all who wish to possess this valuable contribution to the knowledge of the mycopathology of animals.

C. J. LA TOUCHE

Gasteromycetes—Houby-Břichatky. Flora ČSR. Řada (mykologicko-lichenologická) B. Svazek I. By K. CEJP, Z. MORAVEC, A. PILÁT, Z. POUZAR, V. J. STANĚK, M. SVRČEK, S. ŠEBEK and F. ŠMARDA; ed. by A. PILÁT (Czech text with Latin summary). Prague: Czechoslovak Academy of Sciences, 1958. Pp. 864, 256 plates, line-drawings and distribution maps. Price: 87.50 Kčs. (Czechoslovak crowns).

Vol. 1 (Gasteromycetes) of series B (Mycology and Lichenology) of the Flora of the Czechoslovak Republic heralds a considerable advance in the knowledge of the European gasteromycetes.

Regional monographs have appeared in recent years for most parts of the globe; Europe is no exception and, since Hollós (*Die Gasteromyceten Ungarns*, 1904), monographs have appeared for Italy (Petri), Norway (Eckblad), Portugal (Lima Carneiro), Roumania (Alexandri), Sweden (Th. C. E. Fries) and the Canton Ticino in Switzerland (Benzoni). Hawker, Knapp, M. Lange and Soehner have produced revisions of the hypogeous gasteromycetes for Britain, Switzerland, Denmark and Bavaria, respectively, and there have been minor monographic and taxonomic works. The systematics of the group are now reasonably stable with most workers generally accepting the higher taxonomic divisions, but many problems remain to be solved.

Many gasteromycetes are typical fungi of sandy areas, and the occurrence of numerous steppe species in Czechoslovakia was to be expected. The *Flora ČSR* contains descriptions of 57 genera, 249 species, 120 varieties (including 40 typical varieties, of which 5 are as yet unknown for the country) and 28 forms (including 9 typical forms), whilst a further 12 genera and 25 species are to be found in the Czech keys. 38 genera, 137 species, 58 varieties and 18 forms are known for Czechoslovakia with 7 species, 25 varieties and 9 forms endemic; 20 species, and 8 varieties are only known outside Europe. In Great Britain we have 33 genera and about 94 species and 4 varieties. With about 50% of the taxa unknown in Czechoslovakia, the contents would appear to belie the title, but the introduction states that all known European and some non-European gasteromycetes have been embraced as the country is far from thoroughly worked, with new records occurring annually. The monograph can, therefore, be considered a basis for a more thorough study of the Czechoslovak (and European) gasteromycetes and, in this role, will be of exceptional value to workers in the group.

The editor, Dr Pilát, has also prepared the introduction, the chapters on classification, the list of authors (which includes many brief biographies) and of general literature, the indexes of Czech popular and Latin names, as well as the sections dealing with the Phallales and Gauteriales, and the general account of the Sclerodermatales, of which he has dealt with the families Calostomataceae, Glischrodermataceae and Pisolithaceae. The remaining groups are by Prof. Cejp (Nidulariales), Dr Moravec (Arachniaceae, *Disciseda* and Tulostomataceae, excluding *Tulostoma*, for which Dr Pouzar is responsible), Dr Staněk (Astraeaceae and Geastraceae), Dr Svrček (Hymenogastrales, Hysterangiales and Melanogastrales), Mr Šebek (Podaxales, Mycenastraceae and Sclerodermataceae) and Dr Šmarda (Lycoperdaceae excluding *Disciseda*).

The classification is mainly traditional, being modified from Zeller (*Mycologia*, **41**, 36–58, 1949). Following Malençon (*Rev. Mycol., Paris*, **20**, 81–130, 1955), the group, which is treated as a Class, is divided into the Subclasses Exogasteromycetidae (Phallales, Hysterangiales, Hymenogastrales, Gauteriales, Gastrosporiales and Podaxales) and the Endogasteromycetidae (Lycoperdales, Melanogastrales, Sclerodermatales and Nidulariales).

A total of 65 new taxa is described: two orders (Gastrosporiales and Melanogastrales), one genus (*Vascellum*), 12 sections, 4 subsections, 9 stirps, 5 species (*Hymenogaster vacekii*, *Geastrum hollosii*, *G. kotlabae*, *Tulostoma kotlabae* and *T. moravecii*), 22 varieties and 10 forms. There are also 62 new combinations and 2 new names (*Hymenogaster submacrosporus* Svrček replacing *H. macrosporus* Knapp & Soehner and *Hysterangium pseudostoloniferum* Svrček replacing *H. stoloniferum* sensu Hesse).

Workable keys are given to all levels of taxa. The Czech keys are brief, full formal diagnoses being given separately for all except a few non-European taxa. However, the Latin keys (which account for the major part of the Latin text, pp. 703–827) contain full taxonomic, ecological and other data.

The Czech language species format commences with the scientific name and the Czech translation, followed by the vernacular name (sometimes several) in Polish, German, French, English, Russian and even Japanese!), synonymy, formal diagnosis, ecology, type locality, distribution and full data of Czechoslovak collections (sometimes with a full page distribution map), followed by observations and discussion. In both the Czech diagnoses and Latin keys, Czechoslovak species are printed in large type whilst all lower taxa and non-Czechoslovak species are in small type.

In addition to the list of general literature, separate references are given for the Phallales, Podaxales and Nidulariales, as well as for most of the families in the remaining orders. Irregularities in these lists are the different transliterations of the cyrillic and the positions of Czechoslovak names commencing with diacritical characters, often a point of confusion for foreigners. In the Phallales (p. 46) 'Šimr, Šmaha' etc. follow 'Svoboda', whilst in the Geastraceae (p. 398) they appear before 'Smith', and there are similar occurrences with other typical Czech symbols.

The hypogea (Gastrosporiales, Gauteriales, Hysterangiales, Hymenogastrales and Melanogastrales) number 124 species, 24 varieties and 2 forms, of which only 52 species, 21 varieties and the 2 forms have been recorded for the country, and two typical varieties are unknown. In contrast, Dr Hawker records for Britain 27 species and 2 varieties, most of which are also known in Czechoslovakia. *Octaviania* is preferred to *Arcangeliella*, with *O. asterosperma* as the type species, though Zeller & Dodge (*Ann. Mo. bot. Gdn*, **23**, 599–638, 1936), considered *Octaviania* to be based on material of *Melanogaster*. No mention is made of *Wakefieldia* Corner & Hawker; *W. macrosporus* is placed under *Sclerogaster*.

The erection of the new genus *Vascellum* for *Lycoperdon hyemale* Bull. em. Vitt. (= *L. depressum* = *L. pratense* sensu Lloyd) was not unexpected as, with the species being intermediate between *Calvatia* and *Lycoperdon* but distinguishable from both by the diaphragm between the gleba and subgleba, this course was suggested by Perdeck (*Blumea*, **6**, 480–515, 1950). The introduction of *Lasiosphaera* Reich. for the giant puffball (*Calvatia gigantea*) is far less welcome. The difference between many genera of the Lycoperdaceae is so slight that, though they provide useful niches, it seems undesirable to increase their number unduly. Also, the spelling is too close for comfort to the pyrenomycete genus *Lasiosphaeria* Ces. & de Not. *Bovista hungarica* is replaced by the earlier *B. graveolens* Schwalb.

The Geastraceae have received the most extensive treatment and occupy 135 pages of the Czech text. Dr Staněk has created new sections, subsections, stirps, 2 species, 12 varieties and 6 forms. The genus *Geastrum* is exceptionally plentiful in Czechoslovakia, but a surprising absence is *Myriostoma*, though it is present in adjacent countries. One cannot help feeling doubtful about the multitude of minor taxa, especially in view of the variability of these fungi. *Trichaster* is treated as a synonym of *Geastrum*.

Scleroderma is an illustrated revision of Mr Šebek's earlier English language monograph of the Central European species (*Sydowia*, **7**, 158–190, 1953). Whilst many workers may not favour his stress on gross morphology for specific delimitation in this extremely polymorphic genus, the transfer of his former ssp. *bovista* as *S. aurantium* var. is welcomed.

The treatment of the Nidulariales is traditional, but species such as *Cyathus fimetarius* DC and *Nidularia granulifera* (Holm. ex Nees) Fr., which have not been known with certainty since classical times, would have been better placed under 'Doubtful Species', as has been done with certain of the hypogea. The recombination of *N. granulifera* with *Nidula* on the grounds that it may belong to this genus adds yet another name and the use of *Nidularia pulvinata* (Schwein.) Fr. for certain forms would appear to be on descriptive grounds rather than on actual comparison with North American material.

Newly described taxa not yet collected in Czechoslovakia are *Geastrum hollosii* and *G. kotlabae* (both from Hungary), seven varieties, viz. *Myriostoma coliforme* var. *capillisporum* (South Africa), *G. coronatum* Pers. var. *muelleri* (Hungary), *G. lageniforme* var. *ahmadii* (Pakistan and South Africa), var. *koreanum* (Korea) and var. *umbrinum* (Hungary), *G. saccatum* var. *cinereum* (Germany) and *Astraeus hygrometricus* var. *koreanus* (Korea), and one form, *G. triplex* f. *pedicellatum* (France). Their publication would have been preferable in a major language journal of the region where collected, especially for the extra-European material.

A number of misprints appear in the text but more serious are the several valid names, normally printed in bold type in the index, which appear in italic, whilst several taxa are printed in the wrong type in the Latin section. A surprising omission from the Latin keys is *Geastrum lageniforme* var. *umbrinum* which, in the absence of a Latin diagnosis, is not validly published, and other taxa are absent from Czech or Latin sections.

In the main, the authors have observed the International Rules of Botanical Nomenclature, but there are instances of deviation, as in the use of Czech diacritical characters, i.e. *Geastrum Šmardae*, and vowels in epithets derived from proper names which directly contravene Article 73 of the Code. One can, however, sympathize with the Czechoslovaks for wishing to maintain their pronunciation, especially if their probable attempt at *Hymenogaster thwaitesii* is imagined!

The volume is fully bound in linen. The figures and photographs vary in quality, some being really excellent, especially the glossy plates showing up to 105 individuals of geasters of all shapes and sizes. The emphasis tends to be on macroscopic rather than microscopic characters.

The use of Czech, a minor and one of the most difficult of the Slav languages, for a work of major importance is to be regretted as, even with its amplified Latin keys, the Czech discussion of taxa will undoubtedly limit its full use to those familiar with the Czech tongue. This applies not only to western Europe but also to many central Europeans. Even a Russian colleague writes that he finds Czech far from easy to read!

The price is 87.50 Kčs. and the current rate of exchange is about 20 Czechoslovak Crowns to the £. The volume is, however, obtainable in this country from the sellers of Eastern European publications (Collett's Holdings, Ltd., London) for 66*s.* and is very well worth the price. The Czechoslovaks are to be congratulated upon this first volume and we can now look forward to volume II of the Series, *Oomycetes I* by Prof. Cejp, which is stated to be in the press.

J. T. PALMER

A Guide to the Study of Lichens. By URSULA K. DUNCAN. (Arbroath: T. Buncle and Co., 1959). Pp. xxvii+164, 19 plates. Price 25*s.*

This work is described in the introduction as intended for the use of botanical students with no previous knowledge of the subject and as an attempt to co-ordinate in simplified form for the convenience of beginners the various works produced in recent years, but it will be of undoubted value to those who already have some experience of lichenology, including, as it does, much information under *Cladonia* and other critical genera. The classification and, with a few exceptions, the nomenclature are based upon the *Census Catalogue of British Lichens* by W. Watson.

The introductory part dealing with the structure, microscopic examination, ecology, etc. is especially well written, but its value to the beginner would have been enhanced by the inclusion of a few figures illustrating the structure of the thallus, soralia, the types of ascocarps, etc. The main part of the work comprises keys to the Families and Genera and a simple description of one common species of each genus followed, in smaller type, by a brief, more technical description of the remaining species. It is to be regretted that it had not been possible, presumably on account of the cost, to include a line drawing of each chosen 'common' species of the macrolichens, since these are so frequently found sterile and cannot be readily identified with the aid of the keys. The 43 species represented on the plates, however, go far to remedy this deficiency. A reasonably full bibliography is given. The author is to be congratulated on this useful addition to the British lichen literature, doubly welcome in view of the recent revival of interest in the group.

A. E. WADE

Studies on Cercospora *and allied genera.* I. Cercospora *species with coloured spores on* Phyllanthus (*Euphorbiaceae*). By F. C. DEIGHTOH. (Kew, Surrey: Commonwealth Mycological Institute [Mycological Papers, No. 71], 1959.) Pp. 23, 13 text-figures. Price 9*s.*

In addition to the single species on *Phyllanthus* included in Chupp's Monograph (*Cercospora phyllanthi*), five other species (three new) are described, with notes on two species on related genera.

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